CHEMISTRY OF NATURAL PRODUCTS

RESUME

Ph.D. THESIS
IN
CHEMISTRY

ALIGARH MUSLIM UNIVERSITY, ALIGARH

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RESUME

Plants have always been a common source of medicaments either in the form of traditional preparations or as pure active principles. It is mainly during the last 100 years that some of the active ingredients present in herbal prescriptions have been isolated and introduced into **"MODERN MEDICINE"**. There are atleast **119 distinct chemical substances** derived from plants that can be considered as important drugs currently in use. A few of the drugs are simple synthetic modifications of naturally occurring substances. In some instances, the natural products have recently been replaced by commercially available synthetic products. Thus drugs derived from plants still occupy an important position.

In the present studies we have carried out systematic chemical investigation of seven important indigenous medicinal plants and isolated and elucidated the structures of a number of compounds. These products may be helpful to other researchers who are mainly concerned with the biological and clinical aspects of the herbal drugs.

The present thesis is based on **seven chapters**. *Each chapter is devoted to one particular medicinal plant on which the present detailed chemical investigations have been carried out. In each chapter, area of distribution of the plant alongwith its different vernacular names, therapeutic uses of the plant in different traditional systems of medicine and an exhaustive review of the scientific work reported in literature have been given.*

In the beginning of the thesis in the part of **"Introduction"**, *the importance of medicinal plants in general has been highlighted and the biological importance of the two groups of compounds, namely triterpenoids and flavonoids has been reviewed, the reason being that the compounds which we isolated and characterised are mostly flavonoidal and triterpenic in character.*

CHAPTER-I

STUDY OF THE WHOLE PLANT OF *CORCHORUS DEPRESSUS* (LINN) SYN., *C. ANTICHORUS* (RAEUSCH) OR *ANTICHORUS DEPRESSUS* (LINN) (N.O. TILIACEAE)

The dried plant material was chopped into small pieces and exhaustively extracted with boiling ethanol. The ethanolic concentrate on leaving in a refrigerator deposited a solid which was filtered and coded as "A". The filtrate was evaporated to dryness and the residue extracted

successively with(i) petroleum ether(60-80°) to give E-1; with (ii)chloroform to give E-2 and finally with (iii) acetone to give E-3. E-2 was further resolved into neutral and acidic fractions (F-1) and (F-2) respectively. E-3 was dissolved in water and filtered to give the water soluble(W-S) and water insoluble (W-INS)fractions. The water insoluble fraction (W-INS) was dissolved in boiling ethyl acetate. After concentrating the solution to a small volume and leaving in the fridge, a solid "B" separated out which was filtred. The filtrate was evaporated to dryness and coded as "BM". Usual work up and purification of all the above extracts/fractions gave different compounds which are listed in the following Table-I

TABLE-I

S.No.	Fraction/Extract	Compound
1	"A"	2α, 3ß, 20ß-trihydroxy-urs-Δ^{12}-ene,24, 28 dioic acid.
2.	E-1	ß-sitosterol
3.	F-1	ß-sitosterol-ßD-glucoside
4.	F-2	2α,3ß,20β-trihydroxy-urs-Δ^{12}-ene 24, 28-dioic acid.
5.	W-S	Corchorenic acid (2α,3ß-dihydroxy-urs-$\Delta^{12,20}$-diene 24,28 dioic acid)
6.	B	Depressin (2α,3ß,20ß-trihydroxy-urs-Δ^{12}-ene-24,28 dioic acid 24-ßD-galactoside)
7.	BM	(i) Apigenin (ii) Luteolin

Chorchorenic acid and **Depressin** are **new compounds** and are being reported for the **first time.**

CHAPTER-II

STUDY OF THE LEAVES OF *ICHNOCARPUS FRUTESCENS* (R.Br) SYN., *APOCYNUM FRUTESCENS* (LINN) (N.O. APOCYNACEAE)

The dried leaves were exhaustively extracted with ethyl alcohol. The ethanolic concentrate was extracted with petroleum ether(60-80°) to give fraction (F-1). The petrol insoluble part was taken up in water and separated into water insoluble fraction (F-2) and water soluble fraction (F-3). The fraction (F-2) was extracted successively with chloroform and solvent ether. The water

soluble fraction (F-3) was subjected to liquid-liquid extraction with petrol, solvent ether, ethyl acetate and butanol successively. The petrol and ether extracts (liquid-liquid extraction) did not give any appreciable quantities of the products and were not studied further. Other fractions on purification gave different compounds which are listed in the following Table-II.

TABLE-II

S.No..	Fraction	Compound
1.	F-1	Ursolic acid
2.	F-2(Chloroform)	Ursolic acid
3.	F-2(Ether)	Kaempferol
4.	F-3(Ethyl acetate)	Kaempferol 3-galactoside
5.	F-3(Butanol)	Mannitol

CHAPTER-III

STUDY OF THE AERIAL PARTS OF *SISYMBRIUM IRIO* (LINN) (N.O.CRUCIFERAE)

The dried plant material (aerial parts) was exhaustively extracted with ethanol and the ethanolic concentrate was extracted with petroleum ether(60-80°) to isolate the petrol soluble products. The petrol insoluble residue was taken up in water and filtered to separate into water soluble and water insoluble fractions. The quantity of water insoluble fraction was too less to be studied further. The water soluble fraction was hydrolysed by dilute acid to give an aglycone. All these fractions were purified to give pure products which are listed in the following Table-III.

TABLE-III

S.No.	Fraction	Compound
1.	Petrol soluble fraction	(i) ß-sitosterol (ii) ß-sitosterol glucoside
2.	Aglycone fraction	(i) Isorhamnetin (ii) Quercetin

CHAPTER-IV

STUDY OF THE FLOWERS OF *ACACIA LEUCOPHLOEA* (ROXB) WILLD.SYN., *MIMOSA LEUCOPHLOEA* (ROXB) (N.O. MIMOSACEAE)

Air dried flowers were exhaustively extracted with ethanol and the ethanolic concentrate on extraction with petroleum ether(60-80°) gave a petrol soluble fraction PET-S. The residue left after petrol extraction was dissolved in water and filtered to give water soluble(W-S) and water insoluble (W-INS) fractions. The water insoluble fraction (W-INS) could not be purified inspite of repeated attempts and hence further studies were abandoned. The water soluble fraction(W-S) was subjected to liquid-liquid extraction with petrol(60-80°), solvent ether, ethyl acetate and butanol. The petrol(60-80°), and ether extracts (liquid- liquid extraction) did not yield any appreciable quantity of products to study further. Purification of petrol soluble fraction and products obtained by liquid- liquid extraction gave different compounds which are given below in Table-IV.

TABLE-IV

S.No.	Fraction	Compound
1.	PET-S	(i) Behenic ester
		(ii) ß-sitosterol
2.	W-S(Ethyl acetate)	Quercetin 3-glucoside
3.	W-S(Butanol)	Mannitol

CHAPTER-V

STUDY OF THE FLOWERS OF *DODONAEA VISCOSA* (LINN)(N.O. SAPINDACEAE)

Fresh flowers were exhaustively extracted with ethyl alcohol. The ethanolic concentrate was successively extracted with petroleum ether (60-80°), chloroform, acetone and methanol. The acetone fraction was dissolved in water and filtered. The filtrate on acid hydrolysis gave an aglycone. Similarly methanol extract was dissolved in water and filtered. The aqueous solution on acid hydrolysis gave an aglycone which was successively extracted with chloroform and solvent ether. Some of these fractions on purification gave different pure compounds which were characterised and are given in the following Table-V.

TABLE-V

S.No.	Fraction	Compound
1.	Chloroform extract of the ethanolic concentrate	Penduletin
2.	Acetone-extract (Hydrolysis product)	i) Isorhamnetin ii) Quercetin
3.	Methanol extract (Hydrolysis product)	
	(a) Chloroform soluble part	i) Isorhamnetin ii) Doviscogenin(3ß, 15α,21ß,22α 28-pentahydroxy-16α angeloyloxy-Δ^{12}-oleanene)
	(b) Ether soluble part	(i) Isorhamnetin

CHAPTER-VI

STUDY OF THE MALE FLOWERS OF *LUFFA CYLINDRICA* (LINN) M.J. ROEM SYN., *LUFFA AEGYPTICA* MILL. EX.HOOK F.(N.O. CUCURBITACEAE)

The dried flowers were exhaustively extracted with ethyl alcohol. The ethanolic concentrate was successively extracted with petrol (60-80°) and acetone. The acetone extract on purification gave apigenin.

CHAPTER-VII

STUDY OF THE WHOLE PLANT OF *CORONOPUS DIDYMUS* (LINN) (N.O. CRUCIFERAE)

Shade dried plant material was exhaustively extracted with ethanol. The ethanolic concentrate was successively extracted with petroleum ether(60-80°), chloroform and solvent ether. The solvent ether extract on purification gave a crystalline compound which was identified as chrysoeriol.

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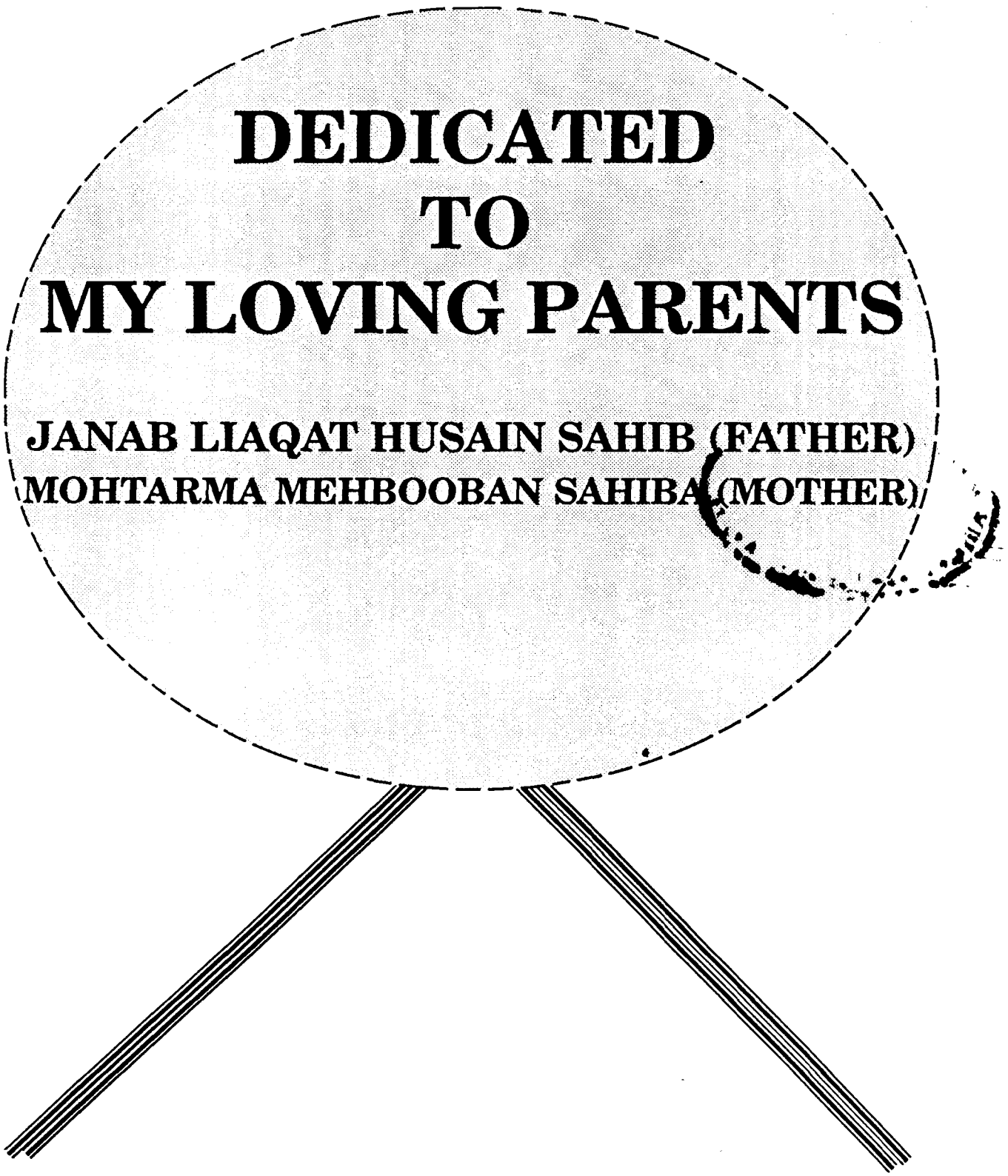
DEDICATED
TO
MY LOVING PARENTS

JANAB LIAQAT HUSAIN SAHIB (FATHER)
MOHTARMA MEHBOOBAN SAHIBA (MOTHER)



CERTIFICATE

It is certified that the research work in this thesis entitled "***Chemistry of Natural Products***" is the original work of the candidate, ***Mr. Kalim Javed***. This work has been carried out under our supervision & guidance and is suitable for submission for the award of the **Ph.D. degree** in **Chemistry**.

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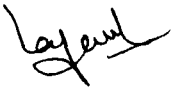

(KALIM JAVED)

The work reported in this thesis ***"Chemistry of Natural Products"*** was carried out in the ***Department of Medicinal Chemistry, Institute of History of Medicine & Medical Research*** (Faculty of Science, Jamia Hamdard) ***New Delhi***, under the direct supervision and able guidance of ***Prof.Dr.M.S.Y. Khan.***

(KALIM JAVED)

CONTENTS

INTRODUCTION

INTRODUCTION

Traditional systems of medicine, which have served Man through the ages to alleviate suffering and disease in various parts of the world, have been recognised by **WHO** as an essential building block for primary health care. One of the chief contributions that traditional medicine has made to health is the discovery and use of plants of medicinal value.[1,2] **Unani-Tibb** (of Graeco-Muslim Origin) and **Ayurveda** are the two forms of traditional medicine most commonly used in India. As a rough estimate the percentage of the population of India using traditional medicine is 60-70.[3]

Plants have always been a common source of medicaments either in the form of traditional preparations or as pure active principles. In a survey done by WHO it has been estimated that 80% of more than 4000 million inhabitants of the world rely chiefly on traditional medicines for their primary health care needs and it can safely be presumed that a major part of traditional therapy involves the use of plant extracts or their active principles.[4] In the developed countries too plant derived drugs are important. In USA, for example, 25% of all prescriptions dispensed from community pharmacies, contain plant extracts or active principles prepared from higher plants.[5,6]

It is mainly during the last 100 years that some of the active ingredients present in herbal prescriptions have been isolated and introduced into "**modern**" medicine. Farnsworth *etal*[4] pointed out in their review article that there are atleast **119** distinct chemical substances derived from plants that can be considered as important drugs current in use. A few of the drugs are simple synthetic modifications of naturally occuring substances. In some instances, the natural products have now been replaced by commercially available synthetic products. Thus the drugs derived from plants still occupy an important position. Some important biological actions of the two groups of naturally occuring compounds viz. triterpenoids and flavonoids are described below:

BIOLOGICAL ACTIVITY OF TRITERPENOIDS

Extensive biological investigations on triterpenoids have revealed a broad spectrum of pharmacological and physiological activities[7] such as antiinflammatory, antineoplastic, anti-bacterial, antifungal, spermicidal, central nervous system acting, diuretic, antidiabetic, metabolite-displacing, molluscicidal, ascaricidal and as antifeedants. A few of the triterpenoids have already gained the status of drugs and are being used clinically.

The triterpenoids of the oleanene and ursene series are active against carrageenan-induced oedema and formaldehyde induced arthritis in rats.[8] It has been observed that the anti-inflammatory activity of the triterpenoids of oleanene series increases with the polarity of compounds which in turn is enhanced by the number of hydroxyl groups present in the molecule.[9] Oleanolic acid 3β- glucoside showed significant activity in the exudative and proliferative phase of inflammation in rats.[10] Ursolic acid, lupeol and taraxerol significantly lowered the incidence of gastric ulceration induced in rats.[11] The triterpenoids, glycyrrhetic acid[12] from *Glycyrrhiza glabra*, boswellic acids[13] from *Boswellia serrata*, bassic acid[14] from *Bumelia sartorum* have been found to exhibit promising antiinflammatory activity.

Tingenone, a triterpene quinonemethide isolated from various plants of *Celestracea* and *Hyppocrateacea* families has displayed antineoplastic activity.[15-17] Cucurbitacins have also received much attention because of their cytotoxic[18-26] and anticancer effects[18-21]. Several other triterpenes including maytenfolic acid[27] from *Maytenus diversifolia*, zeorin and missoruin[28] from *Iris missouriensis* have been reported to possess antineoplastic activity.

Maytenoic (pulpunonic) acid[29], oleanolic acid and ursolic acid[30] are reported to exhibit antibacterial activity. Fusidic acid and helvolic acid, the two tetracyclic triterpenoids are known antibiotics.

Using *Saccharomyces carlsbergenesis* as a test organism, the antifungal activity of forty nine pentacyclic triterpenoids was screened and it was found that the pentacyclic triterpene glucosides of oleanolic acid and hederagenin with a free carboxylic group at C-28 or C-27 possess the highest fungicidal activity.[31]

A new triterpenic acid, 2,3-diacetate of 2α, 3β, 20β-ursΔ^{12}-ene-24β, 28 dioic acid isolated from *corchorous depressus* produced a non-narcotic type of analgesia against acetic acid induced writhing and electrical noxious stimuli in rats. It also has a potent antipyretic effect.[32] Oleanolic acid 3β-D-glucoside showed a significant analgesic activity against thermal stimulus in rats.[10]

The triterpenoids, friedelin, 16α-hydroxy-3-ketoisomultiflorene and 3β-hydroxy 16-ketoisomultiflorene isolated from *antidesma menasu*, displayed diuretic activity in experimental animals.[33]

Tormentic acid[34] and bassic acid[14] have been found to have good hypoglyacemic activity.

Inspired by the close similarity in chemical structures between triterpenoids and steroids, studies were carried out with metabolite-displacing agents involving triterpenoids and it was found that these compounds depress cholesterol absorption.[35,36]

The triterpene chuanliansu isolated from the bark of *Mellia toosendan* is reported to be an ascaricide.[37] The compound is especially welcomed by the paedestrician due to its efficacy and low toxicity.[38]

Azadirachtin, a triterpenoid isolated from *Azadirachta indica* is an effective antifeedant against a wide variety of insects, including those found in USA and works at concentration as low as 0.1 PPM.[39] Deacetylazadirechtinol another triterpene isolated from the fresh fruits of *Azadirachta indica* has been reported to be as potent as azadirachtin in inhibition of insect ecdysis when fed in artificial diet to larvae of the tobacco budworm.[40] Two, tetranortriterpenoids, nimocinolide and isonimocinolides obtained from the fresh leaves of *A. indica* are found to act as insect growth regulators against house flies and mosquitoes.[41]

Trichilin A and B isolated from *Trichilia roka*, are found antifeedants against North American pest insects, the Southern army worm and the Mexican bean beetle.[42,43] Toonacilin and 6-acetoxy toonacilin, isolated from *Toona ciliata*, showed antifeeding activity against *Epilachna verivestis*.[44] Jacquinonic acid, an ant-repellent triterpenoid has been isolated from *Jacquinia pungens*.[45]

BIOLOGICAL ACTIVITY OF FLAVONOIDS

The structure and features of flavonoids considered to be of importance in biological functions are (i) the presence of an extended conjugated resonating system with a carbonyl chromophore(ii) the presence of aromatic hydroxyl groups, which lead to the ability of flavonoids to interact with certain enzyme systems and (iii) their molecular shape which is responsible for their physiological activity due to their similarity in structure to the animal hormones.[46]

The biological function of this group of compounds in man and animal was first suggested by Szent-Gyorgyi[47] in 1938 who reported that the flavonoids present in the citrus peel were effective in preventing capillary bleeding and fragility associated with scurvy. He named this mixture of

flavonoids as vitamin 'P'. A review[48] of the numerous communications in this area which appeared in 1968 established that flavonoids containing free hydroxyls at 3, 3', 4' positions exert beneficial physiological effects (gossypetin being highly active) on capillaries through the following ways: (a) chelate formation with metals, thereby inhibiting oxidation of ascorbic acid, (b) protection of epinephrine by inhibition of the O-methyl transferase enzyme, which is said to be responsible for maintaining capillaries in satisfactory condition and (c) stimulation of the pituitary-adrenal axis.

The action of natural flavonoids on heart was demonstrated[49] as early as 1936. the unsubstituted parent compound, flavone, exerts coronary dilatory activity and is commercially available under the name 'Chromocor'; its combination with rutin and isoquercetain is also a commercial preparation under the name 'Flavo Ce', useful in the treatment of arteriosclerosis.

Several studies[50] on antiinflammatory activity in flavonoids have been conducted during the last about 20 years.

A stable pharmaceuti al composition consisting of neomycin and a topical antiinflammatory flavonoid in a suitable carrier has been patented for the treatment of acne.[51] Another patent was taken on xanthorhamnin as an antiinflammatory agent. It was isolated from the seeds of *Rhamnus infectoria* and recommended for use in ophthalmology, particularly for topical treatment of collyria and in rheumatoid conditions.

The diuretic effect of myricetin, morin and kaempferol in rabbits was observed as early as 1931; the potency increased with increasing number of hydroxyl groups.[52] Flavonoidal glycosides e.g. quercitrin, rutin, kaempferol-3-rhamnoglucoside and myricetin, luteolin, also were found to exhibit diuretic activity.[53]

Spasmolytic effects of 30 flavonoids were studied on rat small intenstine previously treated with $BaCl_2$ as spasmogen.[54] As a rule, the highest activity was shown by aglycones; the activity increased with increasing number of hydroxyl groups. They were most active if the hydroxyls were in positions 7 and 4' in flavones; 3, 7 and 4' in flavonols; 4 and 4' in chalcones; and 7 in flavanones. In glycosides, the spasmolytic activity also depended on the position and nature of the sugar moiety.[55] In a patent, luteolin is characterized with hypochloretic and spasmolytic activity.[56]

The antimicrobial activity of many naturally occuring flavonoids was studied and quercetin was found to completely inhibit the growth of *Staphylococcus aureus* at a concentration of 0.1 mg/ml.[57] Fisetin completely inhibited the growth of *S.albus* at a concentration of 10μg/ml and *S. aureus* at 40μg/ml in liquid broth media. Thus, the antibacterial effect of fisetin was abolished when the double bond between C-2 and C-3 or the OH group on C-5 was removed.[58] Chlorflavonin is the first chlorine containing flavonoid type antifungal antibiotic reported to be produced by strain of *Aspergillus candidus*.[59] Several flavonoids were found to exhibit prophylactic action against fixed rabies virus in mice. Of these quercetin and quercitrin showed significant and rutin promsing activities.[60] Quercetin was viricidal at a concentration of 100μg/ml to human and procine strains of herpes virus and parainfluenze virus, as measured by subsequent infectivity in human cell lines (HeLa cells).

Several flavonoids have been found to be moderately active against laboratory cultures of malignant cells. Eupatin, eupatoretin[61] centaureidin and 6-demethoxy centaureidin[62] are moderately effective against carcinoma of nasopharynx(KB). An antitumorgenic flavanone isolated from the spots of *Sophora* sp. has been patented.[63] The effects of flavonoids have been claimed in the treatment of radiation sickness[64], thyroid disorders[65,66] and gastric and duodenal ulcers.[67]

Some flavonoids have been studied for their antidiabetic activity. It has been found that chromone ring is responsible for manifestation of hypoglycaemic activity, presence of lateral phenyl ring at C-3 of chromone ring as isoflavones is responsible for intensification and prolongation of hypoglycaemic activity and the activity is further affected by substituents and their positions, nature of carbohydrate radical and level of glycosylation of flavonoids.[68]

Although the enzyme inhibitory actions of flavonoids are known since long, but recently some studies have been directed towards ascertaining the relationship between chemical structure and enzyme inhibitory effect. It was found that C-4' substituted flavonoids stimulated the action of indoleacetic acid oxidase and the C-7 hydroxyl group increased this stimulating effect considerably. In 3', 4' - hydroxyls containing flavonoids, on the contrary, an inhibition of enzyme activity occurred.[69]

In the present study, we have tried to carry out systematic chemical investigation of the following important indigenous medicinal plants with a view to characterise their chemical components which could be a starting point for researchers who are mainly concerned with the

pharmacological and clinical aspects of these herbal drugs. It is interesting to note that the main constitutents of these plants were found to be either flavonoidal or triterpenic in character.

NAME OF THE PLANTS

1. *Corchorus depressus* (Linn).
2. *Ichnocarpus frutescens* (R. Br).
3. *Sisymbrium irio* (Linn).
4. *Acacia leucophloea* (Roxb).
5. *Dodonaea viscosa* (Linn).
6. *Luffa cylindrica* (Linn).
7. *Coronopus didymus* (Linn).

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CHAPTER - I

DISCUSSION

STUDY OF THE WHOLE PLANT OF *CORCHORUS DEPRESSUS* (LINN) SYN.,*C.ANTICHORUS* (RAEUSCH) OR *ANTICHORUS DEPRESSUS* (LINN) (N.O. TILIACEAE)

VERNACULAR NAMES

Local Name - Bohphali/Khurand, **Sanskrit**-Bhadani, **Hindi-** Baphuli, **Gujrati**-Bahuphali.

BOTANICAL DESCRIPTION

It is a prostrate, deep-rooted, much branched and perennial herb. Leaves are plicate, flowers are yellow in colour. Capsules (Fruits) are cylindric, straight or curved and four valved. Flowering and fruiting take place during the months of Feb- Aug[1].

DISTRIBUTION

It is found in the drier parts of India.

MEDICINAL PROPERTIES AND USES

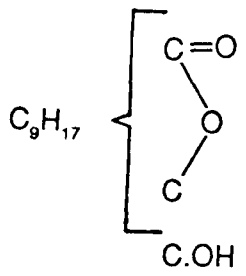
It is used for the treatment of gonorrhoea and its extract is applied as a paste in healing of wounds. The plant has been described to have tonic properties and is given as a cooling medicine in fevers[2]. It is also given to increase the viscosity of the seminal fluid [3].

2, 3-diacetyl derivative of a new triterpenic acid belonging to the α-amyrin series isolated from this plant and characterised as 2α, 3β, 20β-trihydroxy-urs-Δ^{12}-ene-24, 28-dioic acid has been shown to possess antipyretic activity on yeast induced pyrexia at a dose of 100mg/kg. body weight in albino rats. The effect was particularly marked when it was administered intraperitoneally. It also exhibited significant analgesic activity on acetic acid induced writhing in mice at a dose of 100mg/kg. orally and on response to electrical stimulus of mice in pododolorimeter at a dose of 500 mg/kg. orally. However, it was found devoid of analgesic activity against thermal and mechanical stimuli. Toxicity studies showed that it is well tolerated upto 500mg/kg, i.p. in mice.[4]

PAST CHEMICAL INVESTIGATIONS ON *CORCHORUS* SPECIES :-

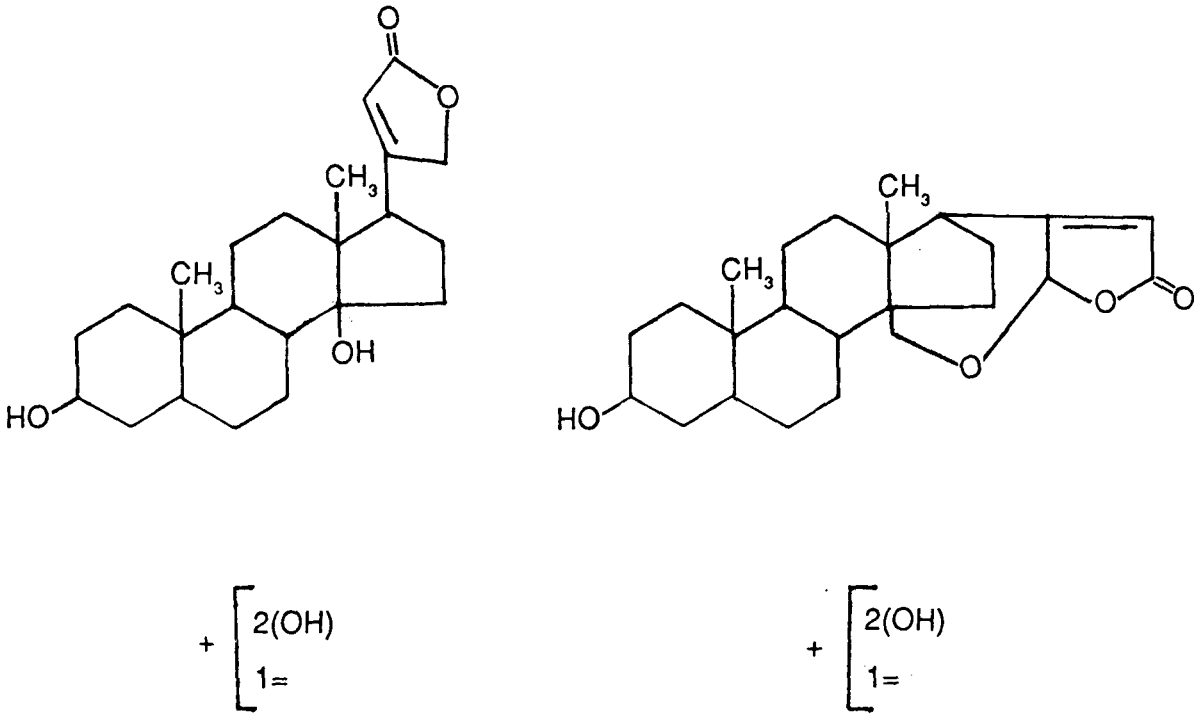
A glycosidic compound named corchorin was isolated from the seeds of *C. capsularis* by Sen, N.K. (1930). Corchorin, on acid hydrolysis (10%, H_2SO_4) yielded corchogenin $C_{16}H_{26}O_3$ and one mole of glucose. On oxidation with $KMnO_4$ it gave glucocorchoric acid (146-48°) which upon acid hydrolysis with dil. HCl, gave glucose and corchoric acid($C_{15}H_{24}O_5$, m.p. 67-70°)[5]. Later on, Soliman and Saleh (1950) isolated a glycosidic compound,($C_{23}H_{32}O_6$ 0.5 H_2O), m.p. 175-77°; $[\alpha]^{18}_D$=40.8 (MeOH) from the seeds of *C. olitorius* which they also named as corchorin.[6] However, it was not identical with corchorin isolated by Sen, N.K.[5] from the seeds of *C. capsularis*. Another glycosidic compound termed as corchorin was isolated from the seeds of *C. olitorius* by Alam *et al.* (1954)[7] and they claimed that it was different from the corchorin reported earlier.[6] Chaudhury and Dutta also isolated corchorin from the seeds of *C. capsularis* and reported it to be a bitter principle.[8] Moslemuddin and Ahmad (1955) carried out the structural studies[9] of corchorin isolated from *C. olitorius*.[7] Corchorin, on acid hydrolysis yielded glucose and corchorogenin which formed acetate, oxime and semi carbazone derivatives[9]. From the above review no definite conclusion can be drawn regarding the chemical structure of corchorin, as different workers have described its different physical and chemical properties.

The glucosidic compound, corchoritin was isolated from the seeds of *C. capsularis*. Corchoritin $C_{12}H_{18}O_3$ (m.p. 218-20°); $[\alpha]^{27}_D$=35.1° (96% EtOH), acetate (m. p. 120-2°); $[\alpha]^{25}_D$=63°,phenyl urethane (m.p 254-66)° is reported to be a laevorotatory compound and has no deoxy sugar. Corchoritin has been reported to have one double bond and it has been assigned the following partial structure[10] **(I)**.



(I)

Cardiac aglycone corchortoxin ($C_{23}H_{32}O_6$), m.p. 247°; $[\alpha]^{18}_D$=67.9° (EtOH), acetate (m.p. 240°) was isolated from the seeds of *C. capsularis*. Corchortoxin was assigned the following two structures[11] **(II & III)**



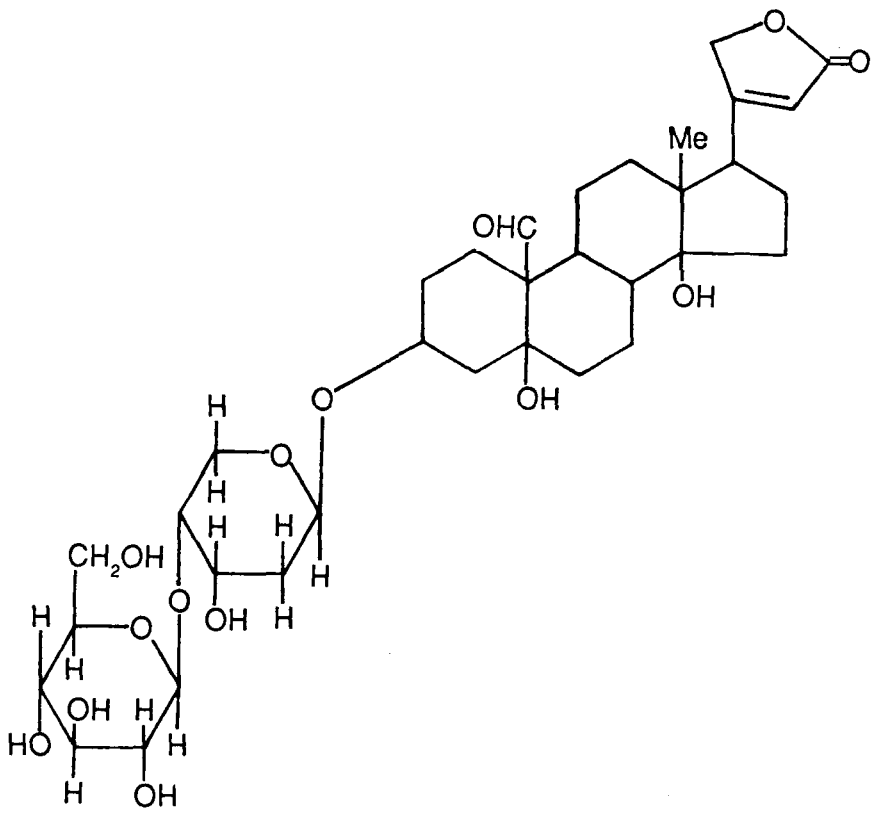
(II) **(III)**

Tow non-crystalline compounds D & E were isolated from the seeds of *C. capsularis*. D was probably the diglucoside of corchoroside A, and E was a triglucoside of corchoroside A. Corchoroside A, on acid hydrolysis yielded D- boivinose and strophanthidin [12].

β-sitosterol and corchorolic acid ($C_{26}H_{52}O_3$, monohydroxy monocarboxylic acid) were isolated from the seeds of *C. capsularis*.[13]

The presence of sugars-raffinose, sucrose, arabinose, fructose, glucose and galactose has been reported in the seeds of *C. capsularis*, While raffinose, arabinose, fructose and glucose are reported in the roots. A new crystalline compound named as corosin (m.p. 290-2°) and β-sitosterol have been reported from the roots[14].

Three glycosides 'A' 'B' and 'C' were isolated from the seeds of *C. capsularis*[15]. Glycoside 'A' was found to be identical with Erysimoside **(IV)** while Glycoside 'C' m.p. 200-10° was not identical with any products reported earlier from *Corchorus* species.



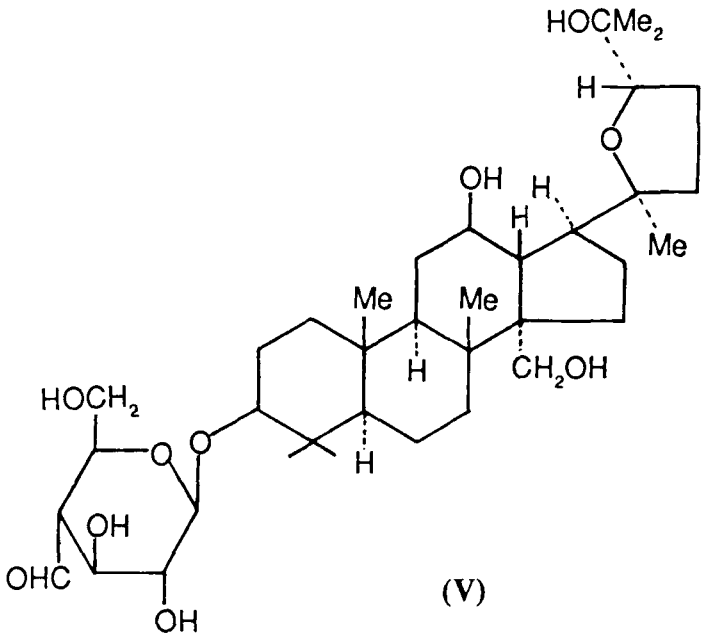
(IV)

A glucoside named capsularin ($C_{22}H_{36}O_8$, m.p 175-6°) was isolated from the leaves of *C. capsularis*. On hydrolysis, it gave glucose and a compound with the molecular formula $C_{16}H_{26}O_3$ (m.p.185°).

β-sitosterol, β-sitosterol-D-glucoside and three glucosides namely, capsulasone (m.p.258-60°); $[\alpha]^{30}_D$=42.8 (EtOH), corchorol- $C_{22}H_{38}O_9$ (m.p.184°); $[\alpha]^{30}_D$=22.6° (EtOH) and capsularol $C_{41}H_{70}O_{11}$,(m.p. 204-5°); $[\alpha]^{30}_D$=20.7 (EtOH) were isolated from the leaves of *C. capsularis*. Beside these, the leaves contained 4% KCl, and small quantities of glucose, galactose & arabinose. Capsularol, on acid hydrolysis yielded glucose and an aglycone capsularo-genin $C_{35}H_{60}O_6$, (m.p. 217°); $[\alpha]^{30}_D$=2.97°(EtOH)[17].

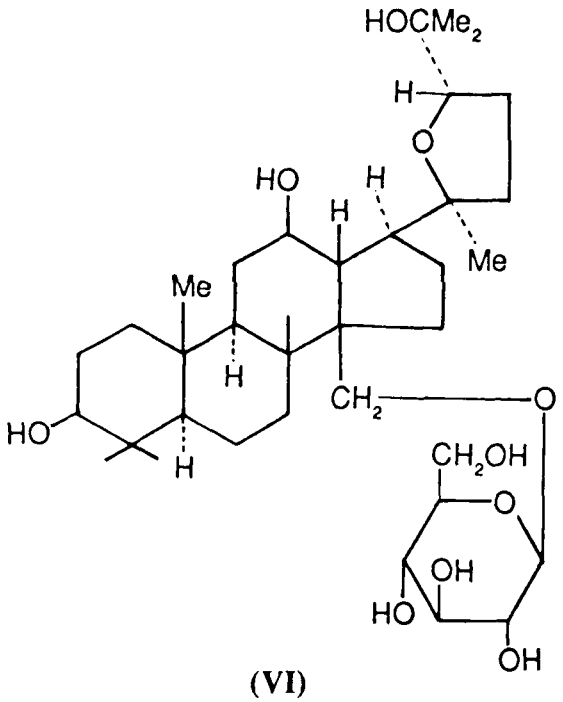
The two compounds, β-sitosterol glucoside, and an unnamed new triterpene glucoside were isolated from the dried leaves of *C. capsularis*. The aglycone of the triterpene glucoside on acetylation gave a diacetate,however, the structure of the aglycone was not determined[18].

From the leaves of *C. capsularis*, a new dammarane triterpene glycoside named as capsin was isolated and characterised as 3- glucoside of 20, 24-epoxy, 3β, 12β, 25, 30-tetrahydroxy dammarane (capsugenin)[19] **(V)**.



(V)

A new compound capsugenin-30-0-glucopyranoside **(VI)** was isolated from mature leaves of *C. capsularis*. Its structure was determined from spectral and chemical data.[20]



(VI)

Cyanidin and its monoglucoside were isolated from the bark while a monoglucoside of cyanidin was isolated from the leaves of *C. capsularis*[21].

The bark of *C. capsularis* contained pectin, KCl, wax (m.p. 85-7°), a white crystalline compound (m.p.276-7°), fructose and galactose. [22].

A new cardio-active aglycone,corchorgenin $C_{23}H_{32}O_6$ (m.p.227°); $[\alpha]^{21}_D$=90° (EtOH), acetate (m.p.240-42°) was isolated from *C. olitorius*. Corchorgenin was found to have a lactone ring and formed isocorchorgenin (m.p. 200°) when kept in 5% MeOH-KOH for three hours at room temperature. The seeds also yielded raffinose (about 2.5%) and a bitter colourless crystalline compound m.p. 172-5°. The pharmacological activity of corchorgenin in cats,indicated that it is more active than either of the isomeric genins, corchortoxin of *C. capsularis* or strophanthidin of *Strophanthus komlee*.[23,24]

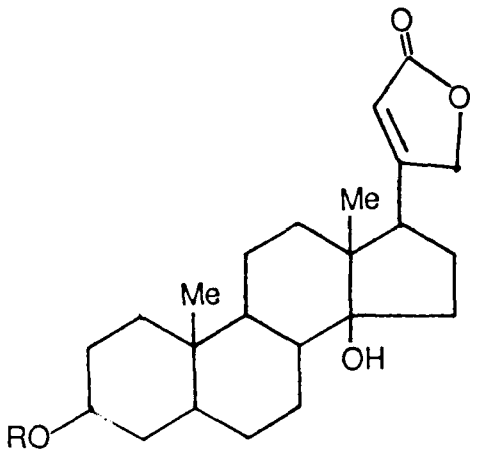
The glycoside A (m.p 178-179°); $[\alpha]^{20}_D$=+11.8°, M.W. 530, was isolated from the seeds of *C. olitorius*[25] which on acid hydrolysis yielded the aglycone strophanthidine. Later on,the structure of glycoside A was resumed which was found to have 2-deoxy-3-methoxy-D-ribose sugar. Another compound, glycoside B was also isolated which was found to have strophanthidin and 2-deoxy-D- ribose sugar.[26]

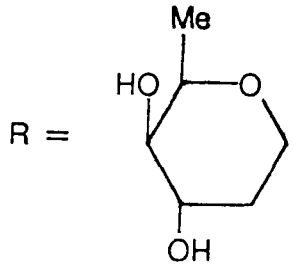
A new glycoside, olitoriside glucoside (m.p.204-6°); $[\alpha]^{22}_D$=4.5°; $C_{35}H_{52}O_{14}$, acetate (m.p. 229°); $[\alpha]^{22}_D$=1.7° was isolated from the seeds of *C.olitorius* which on acid hydrolysis yielded glucose and corchoroside A. Corchoroside A was split by mineral acid to bovinose and strophanthidin. On the basis of these facts, the structure of olitoriside glucoside was established as strophanthidin-3β-D-bovinoside-β-D- glucoside. [27-30].

A glycosidic compound corchoroside A was isolated from the seeds of *C. olitorius* which upon acid hydrolysis yielded strophanthidin and the sugar bovinose[31].

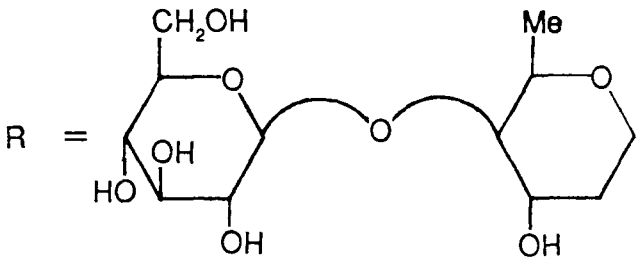
Strophanthidin, strophanthidol, corchoroside A, helveticoside and olitorin were isolated from the seeds of *C. olitorius*.[32]

Two new glycosides coroloside and deglycocoroloside were isolated from *C. olitorius* whose structures were determined as **(VII)** and **(VIII)** according to hydrolysis products and their physical properties.[33]





(VII)



(VIII)

Glucoronic acid, galacturonic acid and pectic acid have been identified in jute fibre.[34]

A new bitter principle named as corchsularin (m.p.158°); $[\alpha]^{25}_{D}$=48.98° (MeOH), diacetate (m.p. 221-3°); $[\alpha]^{25}_{D}$=54.05° (MeOH) was isolated from the seeds of *C. capsularis* and *C. olitorius* which on hydrolysis gave corchsugenin (m.p. 102-3°); $[\alpha]^{25}_{D}$=60.90° (MeOH). The hydrolysate was neutralised with silver carbonate which on concentration gave corchsularose. Several derivatives of corchsularose were prepared and identified as 2-deoxy-3-0-methyl pentose.[35]

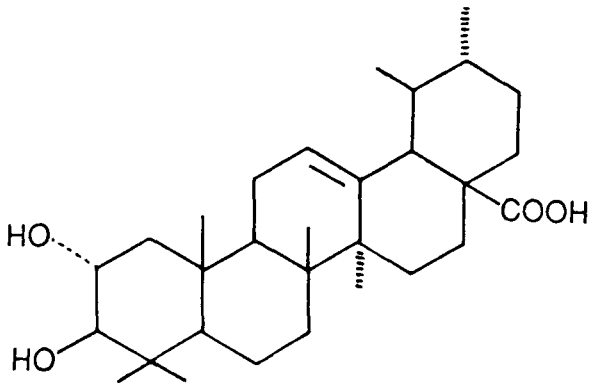
Two new compounds, termed as corchoroside A and corchoroside B were isolated from the seeds of *C. capsularis* and *C.olitorius*. Corchoroside A, $C_{29}H_{42}O_{9}$, (m.p. 188-90°); $[\alpha]^{20}_{D}$=+ 1° (MeOH), acetate (m.p. 166°), on acid hydrolysis yielded a sugar and strophanthidine (oxime m.p.256°); $[\alpha]^{20}_{D}$=+67° (pyridine). The corchoroside B(m.p.222-24°); $[\alpha]^{20}_{D}$=68°, ($C_{29}H_{44}O_{8}$,$2H_2O$) showed all colour reactions of corchoroside A. In addition to this it reacted with periodate and aniline indicating the presence of -OMe[36].

The identity of corchorin, corchorigenin,corchsularin, corchotoxin and strophanthidin was established by direct comparison of these products in the seeds of *C. capsularis* and *C. olitorius*.[37]

Extract of the seeds of *C. olitorius* and *C. capsularis* after enzymic hydrolysis gave fair yields of the corchoroside A while the extract without enzyme treatment gave only a low yield. [38]

A triterpenoid corosin ($C_{30}H_{46}O_7$) m.p. 292-3°; $[\alpha]^{26}_D$=+ 39° (0.5% in MeOH) and β-sitosterol were isolated from the roots of both plants (*C. capsularis* and *C. olitorius*). Corosin gave an acetate ($C_{34}H_{50}O_9$); m.p. 257-8°; $[\alpha]^{25}_D$=4.5 (0.96% in MeOH). When corosin was refluxed with HCl, it gave a new product, corosic acid ($C_{30}H_{44}O_6$); m.p. 247-9°; $[\alpha]^{26}_D$=+127° (0.9% in MeOH). The structure of the corosic acid however, was not established [39].

Ursolic acid, corosolic acid **(IX)** and oxo-corosin were isolated from fresh, undried roots of *C. capsularis* and *C. olitorius*. Their structures were established on the basis of spectral studies[40].



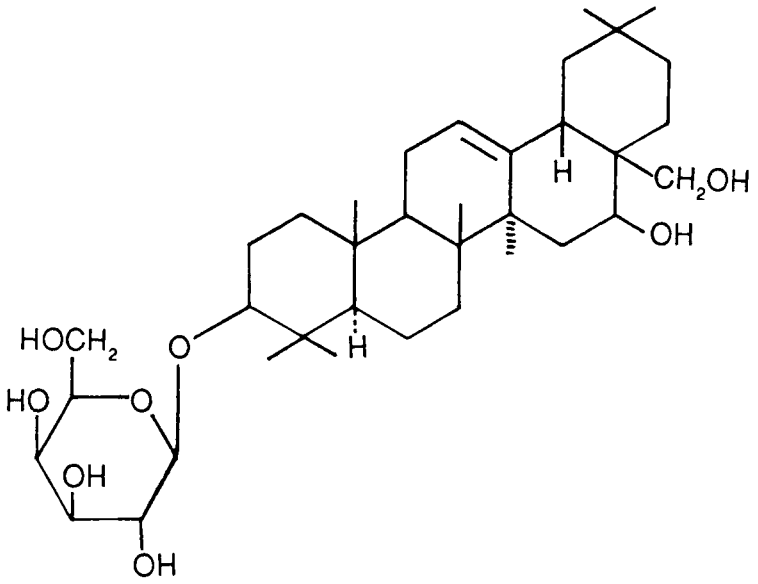
(IX)

The fat/ oil composition of *C. capsularis* (seeds, fibre),[41-44] *C. olitorius* (fibre)[45] and of china jute seeds[46] were also studied.

A flavonol, quercetin was isolated from the fresh plant of *C. acutangulus*.[47]

From the seeds of *C. acutangulus*, helveticoside, corchoroside A, strophanthidine, strophanthidol, digitoxose, boivinose, 2,6-di-deoxy monomethoxy sugar and an unidentified glycoside - A (m.p. 155-60°) were reported and the sugar of glycoside -A was identified as digitoxose[48].

Four new triterpenoid glycosides namely chorchorusins A **(X)**, B, C & D were isolated from the aerial parts of *C. acutangulus* which were respectively defined as longispinogenin-3-O-β-D-galactopyranoside, saikogenin-F-3-O-β-D-galactopyranoside, 23-hydroxylongispinogenin-3-O-β-D-galactopyranoside and saikogenin-F-3-O-β-D-glucopyranosyl (1→2)-β-D. galactopyranoside.[49]

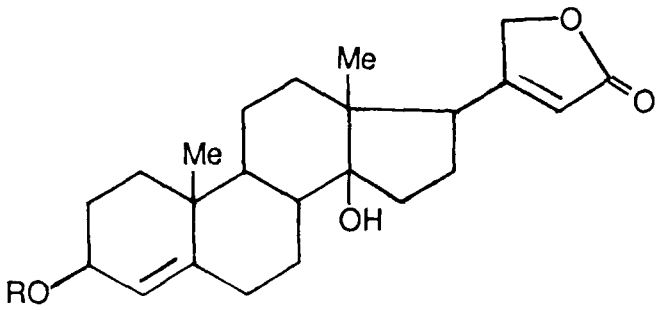


(X)

β-sitosterol and betulinic acid were isolated from *C. fascicularis.*[50]

From the fermented seeds of *C. hirtus*, four strophanthidin hetrosides,namely glycosides a, b, c and d were isolated. The sugar of glycoside c was boivinose and the remaining three glycosides (a, b & d) contained boivinose and glucose[51].

From the seeds of *C. trilocularis*, two crystalline glycosides were isolated. The major glycoside was trilocularin and the minor one was identical with corchoroside B **(XI)**. The structure of trilocularin **(XII)** was determined from chemical and spectral evidences.[52]



(XI) R = α-L-rhamnosyl

(XII) R = 2,6 -dideoxy-β-D-xylo hexopyranosyl.

The flavonoids, quercetin and kaempferol were identified in the leaves and flowers of *C. depressus*[53]

PRESENT WORK

The dried plant material, which consisted of whole plant was purchased from two sources, namely, Kharibaoli market, Delhi and from M/s Hamdard Dawakhana (Wakf) Delhi. The authenticity of the two samples was established in the Department of Botany, University of Delhi, and in the pharmacognosy section of our Institute. Both samples were subjected to identical extraction procedure and both gave very similar results. The plant material purchased from khari baoli was chopped into small pieces and exhaustively extracted with boiling ethanol. The solvent was recovered and concentrated to a small volume. On leaving in the refrigerator, a solid product "**A**" separated out which was filtered, washed with ethanol and dried. The filtrate was evaporated to dryness and the residual mass extracted successively with petroleum ether to give extract **E-1**; then with chloroform to give extract **E-2** and finally with acetone to give extract **E-3**.

STUDY OF PRODUCT "A"

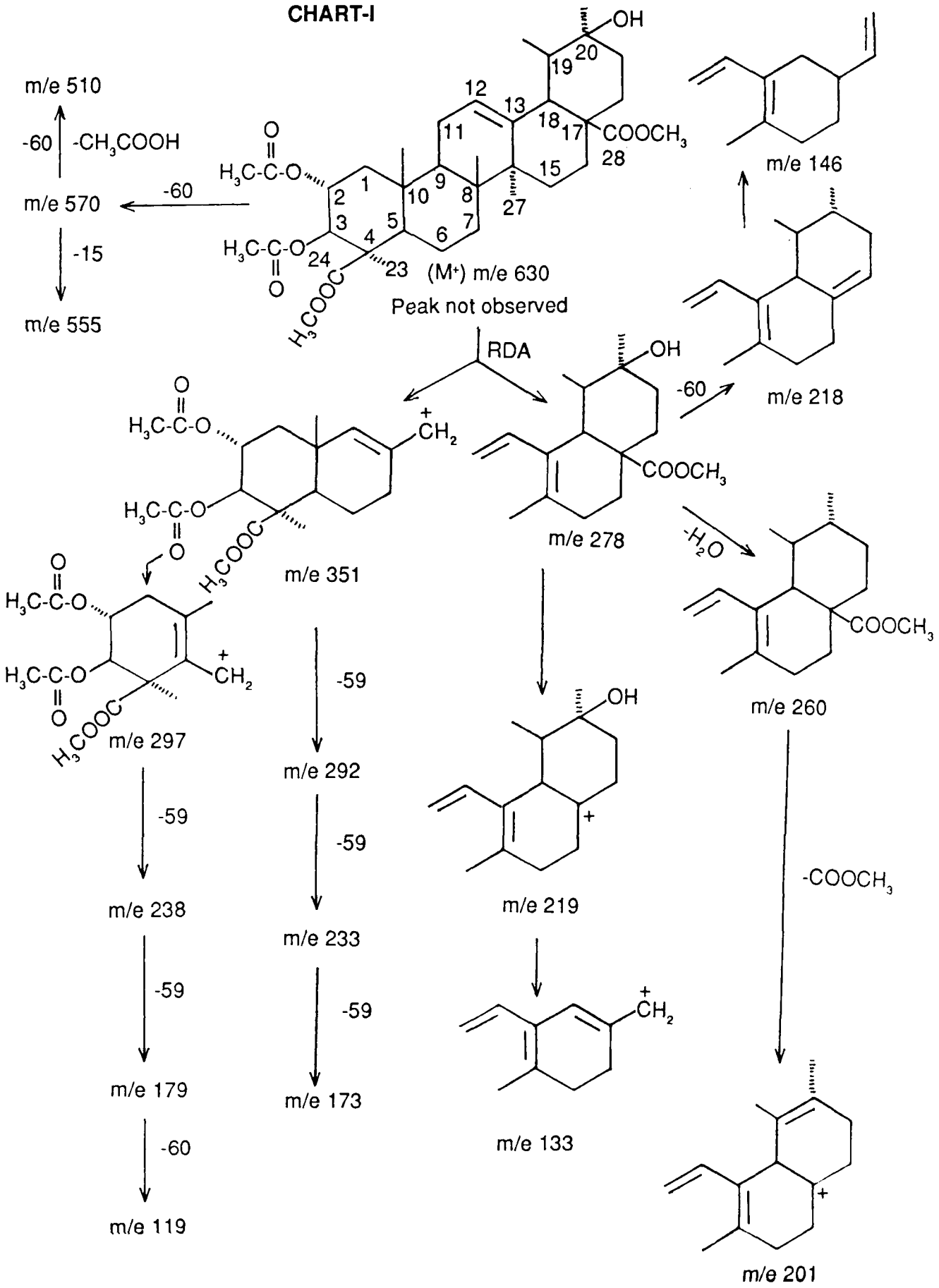
It was extracted by refluxing with petroleum ether (60-80°) twice in order to remove waxy and other petrol soluble fractions. The residue left after petrol extraction gave a positive Liebermann-Burchard test indicating the presence of triterpenes. It was acetylated with acetic anhydride and pyridine in the usual way to give an acetate, m.p. 262-63° **(AA)**. On methylation with diazomethane the acetate gave an acetyl methyl ester, m.p. 185-86° **(AAM)**.

The NMR spectrum of the acetyl methyl ester showed signals for six methyl groups at δ 0.629(s, 3H,), δ 0.849(s,3H), δ 0.894(d,3H), δ 1.15(s,3H), δ 1.209(s,3H), δ 1.217(s,3H), two singlets at δ 1.9297 and δ 2.0299 for two acetyl methyl functions and two ester methoxyl signals at δ 3.65 and δ 3.75. In the low field region, there was a triplet centred at δ 5.288 characteristic of a C-12 proton in the Δ^{12} triterpenes, a doublet at a δ 4.75 for H-3 proton α to the acetoxyl and a doublet of triplet at δ 5.66 for H-2 proton α to the other acetoxyl. The mass spectral fragmentation pattern **(Chart I)** of the compound was the same as reported earlier [54] for the diacetyl dimethyl ester of 2α, 3β, 20β, - trihydroxy -urs Δ^{12}-ene 24, 28-dioic acid and was in agreement with the NMR data also.

In order to substantiate further the ^{13}CNMR spectrum of the parent compound in DMSOd_6 was recorded during the present investigation. The parent compound,m.p. >360° was obtained by deacetylation of the acetate **(AA)**.

The spectrum confirmed the essential conclusions reached already. In the low field region two signals at δ 178.5 and δ 180.0 arose from the two carbonyl carbons of the two COOH functions. In the olefinic region there were two signals at δ 127.5 and δ 139.5, of which the former split into a doublet in the off - resonance decoupled spectrum while the other remained a singlet. These are the characteristic features of oleanenes and ursenes, the former signal arising from C-12 and the other from C-13. In the region from δ 65-δ 85 characteristic of carbinyl carbons, there were three signals at δ 67.0, δ 72.0 and δ 82.5 of which the first and the third arose from secondary carbinyl carbons while the middle one from a carbon carrying a tertiary hydroxyl function. Normally the C-3 of oleanenes and ursenes carrying a 3-OH resonates in the region of δ 78.0-δ 80.0. It shifts to around δ 83.0 in presence of a C-2 hydroxyl, while the C-2 carbon in such systems is observed around δ 68.0, hence of the three signals mentioned earlier, δ 67.0 is assigned to C-2 and δ 82.5 to C-3, thus confirming the presence of 2,3-diol system. The

CHART-I


m/e 510
-60
-CH3COOH
m/e 570
-60
-15
m/e 555
OH
COOCH3
28
27
(M+) m/e 630
Peak not observed
RDA
m/e 146
m/e 218
-60
m/e 278
-H2O
m/e 260
m/e 351
m/e 297
m/e 292
m/e 233
m/e 173
m/e 238
m/e 179
m/e 119
-59
m/e 219
m/e 133
-COOCH3
m/e 201

remaining signals at δ 72.0 can be assigned to C-20 which carries the tertiary hydroxyl.In the low field end of the aliphatic region, two signals at δ 53.5 and δ 55.5 arising from non-oxygenated secondary carbons(appearing as doublets in off resonance) fit for C-5 & C-18 respectively. The two sharp signals at δ 47.0 and δ 49.5 arose from two quaternary carbons. A comparison with literature data[55], suggested that these arise from C-4 and C-17, both of which carry carboxyl functions. In the high field side, six signals could be recognised as arising from methyl carbons and these were located at δ 14.5, δ 15.5, δ 15.7, δ 23.5, δ 24.0 and δ 26.0 which can be assigned respectively to C-25, C-23, C-26, C-27, C-29 and C-30. Of these distinction between C-23 and C-26 and between C-27 and C-29 is made difficult due to closeness of chemical shifts. A complete analysis of the methylene region was not possible because of overlap with solvent signals.

These observations are in perfect accord with the structure proposed as **2α, 3β, 20β-trihydroxy-urs-Δ^{12}-ene, 24,28 dioic acid.**

STUDY OF THE PETROL EXTRACT E-1 (ISOLATION AND CHARACTERISATION OF β-SITOSTEROL)

The petrol was evaporated off from the extract and the residue chromatographed on a column of silica gel. The column was successively eluted with petroleum ether, petroleum ether-benzene mixture (1:1), benzene, acetone and finally with alcohol. The petrol-benzene fraction gave a compound, m.p. 87° which was found to be a mixture and it was therefore rechromatographed on a column of silica gel. The column was eluted with petroleum ether, petrol-benzene mixtures, benzene and finally with acetone. The petrol-benzene(3:1) eluate gave a colourless compound, m.p. 137° which gave a positive Liebermann- Burchard test for steroids. On acetylation with acetic anhydride and pyridine, it gave an acetate, m.p. 126°. On the basis of physical properties, the parent compound was identified as **β-sitosterol** and the identity confirmed by co-T.L.C., co-IR and mixed melting point and a similar comparison of the acetate with authentic β-sitosteryl acetate.

STUDY OF THE CHLOROFORM EXTRACT (E-2), SEPARATION INTO NEUTRAL(F-1) AND ACIDIC (F-2) FRACTIONS

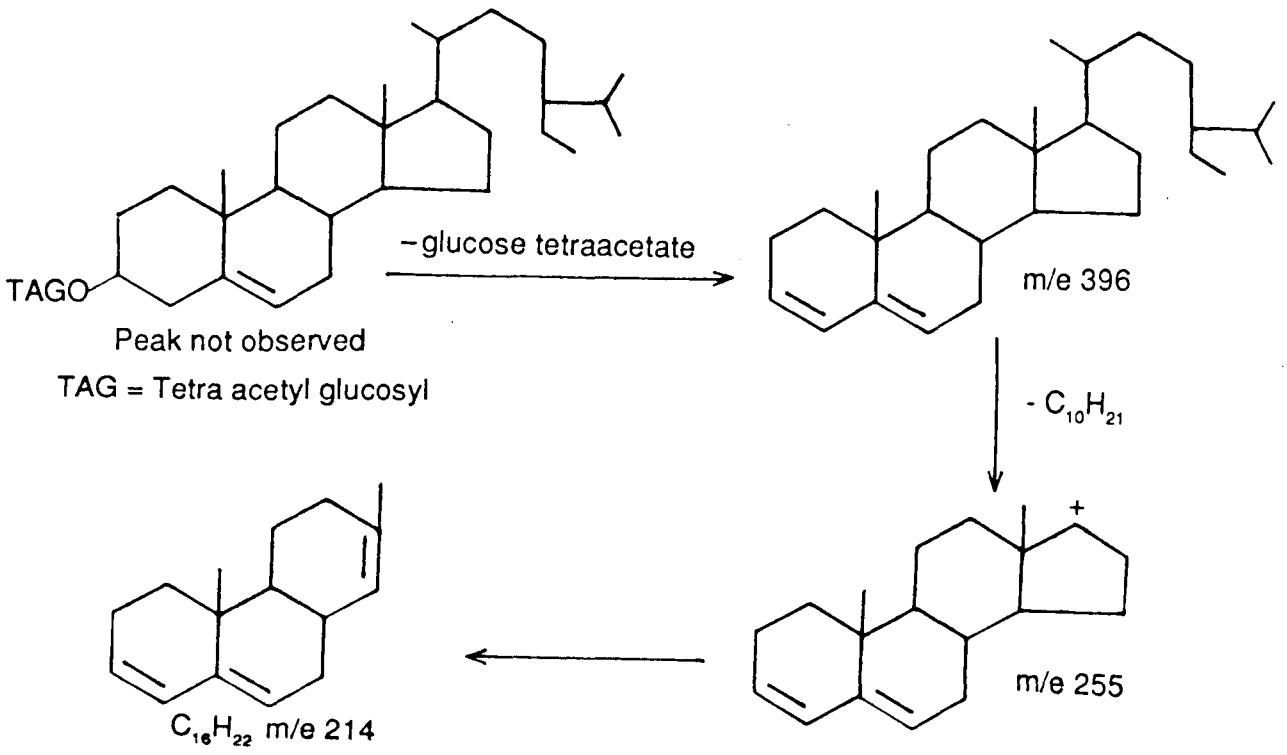
The chloroform extract was evaporated to dryness. The residue was stirred with 4% NaOH solution, heated on a boiling water bath for one hour, allowed to cool at room temperature and

then left in the refrigerator overnight. The insoluble residue was filtered and washed with water. It was labelled as the neutral fraction **(F-1)**. The filtrate on acidification gave a brown precipitate which was filtered and washed with water. It was labelled as acidic fraction **(F-2)**.

STUDY OF THE NEUTRAL FRACTION (F-1), ISOLATION AND CHARACTERISATION OF β-SITOSTEROL GLUCOSIDE

The neutral fraction was acetylated with acetic anhydride and pyridine to give a colourless acetyl derivative, m.p.166°. It gave a positive L.B. test and was T.L. C.pure.The NMR spectrum of the compound showed six methyl functions in the range of δ 0.717 to δ 1.012. The presence of four acetoxyl groups at δ 1.956, δ 1.999, δ 2.020 and δ 2.044 suggested it to be a monoglycosidic acetate. There was a signal centred at δ 5.4 characteristic of an olefinic proton of steroids. It showed a group of multiplets from δ 4.2 to δ 5.1 for protons α to acetoxyls reminiscent of monoglycosidic acetate. From these data it could be concluded that the compound is a monoglycoside of a sterol. Similar conclusions were drawn from the mass spectral data also. The mass spectrum of the glycoside acetate showed the fragmentation pattern characteristic of β-sitosterol **(Chart-II)**.

CHART - II



DEACETYLATION OF THE ABOVE ACETATE

The acetate was refluxed with methanolic sodium hydroxide and poured into water. It was processed as usual to give the crude deacetylated product which crystallised with methanol to give a colourless compound, m.p. 282°.

HYDROLYSIS OF THE ABOVE DEACETYLATED PRODUCT (m.p.282°) BY KILLIANI'S MIXTURE

It was hydrolysed by Killiani's mixture and the contents after diluting with water were extracted by solvent ether. Recovery of ether gave a compound, m.p. 137°, which had identical behaviour as β-sitosterol on T.L.C. and did not show any depression in m.p. on admixture with β-sitosterol.

IDENTIFICATION OF THE SUGAR

The aqueous layer left after ether extraction was evaporated to dryness in a china dish on a water bath and after dissolving the residue in a few drops of distilled water, chromatographed on Whatmann filter paper no. 1 for the presence of sugar along with authentic samples of different sugars. The spots of the sugars were developed by spraying the chromatogram with aniline hydrogen phthalate and warming it in an oven. The spot of the test material corresponded well with glucose and hence the sugar was identified as glucose.

On the basis of the above data, the compound, m.p. 282° was identified as β-D glucoside of β-sitosterol and its acetyl derivative, m.p. 166° was identified as the tetraacetate of **β-D-glucoside of β- sitosterol**.

STUDY OF THE ACIDIC FRACTION, F-2(ISOLATION OF COMPOUND "AA")

This fraction gave a positive L.B. test for triterpenoids and was acetylated with acetic anhydride and pyridine in the usual way to give a crude acetyl derivative which on crystallisation with methanol gave colourless crystalline compound m.p. 260°. On the basis of physical

properties, NMR spectral data and direct comparison with **"AA"**, it was identified as **2α,3β, 20β-trihydroxy-urs-Δ^{12}-ene-24,28-dioic acid** and confirmed by preparation of its dimethyl ester (m.p. 185-86°).

STUDY OF THE ACETONE EXTRACT (E-3), SEPARATION INTO WATER SOLUBLE (W-S) AND WATER INSOLUBLE (W-INS) FRACTIONS

The acetone concentrate was dissolved in water and filtered to give the water soluble and water insoluble fractions. The water soluble part was coded as **(W-S)** and the water insoluble part was coded as **(W-INS)**.

HYDROLYSIS OF THE WATER SOLUBLE PART (W-S), ISOLATION AND CHARACTERIZATION OF A NEW TRITERPENIC ACID NAMED AS CORCHORENIC ACID

The water soluble part was hydrolysed by dil. H_2SO_4 to give a crude aglycone which on acetylation with acetic anhydride and pyridine, gave an acetyl derivative, m.p. 260-63°. The T.L.C. behaviour of the acetate showed it to be slightly contaminated with other impurities, and hence it was deacetylated by refluxing with methanolic NaOH. The reaction mixture on pouring into water did not give any insoluble product, however, on acidification with HCl it gave a colourless precipitate which was washed with water and dried. The filtrate was extracted with ether several times and the ethereal extracts were combined together, washed with water and dried. Recovery of ether left a small residue which was combined with the above precipitate after T.L.C examination. On crystallisation with methanol, it gave T.L.C. pure, colourless, hexagonal crystals, m.p. 295° which on acetylation gave an acetyl derivative, m.p. 277-78°. On methylation with diazomethane, it gave an acetyl methyl ester, m.p.238°.

The NMR spectrum of the acetyl methyl ester showed two acetoxyls at δ 1.97 and δ 2.04 and two ester methoxyls at δ 3.59 and δ 3.67 indicating the compound to be dimethyl ester diacetate. In the methyl region it showed four tertiary methyls at δ 0.87, δ 0.96, δ 1.02 and δ 1.25 and a secondary methyl at δ 1.09 and also a vinylic methyl at δ 1.73. Besides these it showed a triplet at δ 5.36 for one proton and a multiplet at δ 5.75 for one proton. The former signal at δ 5.36 could be attributed to the 12-H of the Δ^{12} oleanene and ursene, while the other signal at δ 5.75 may be attributed to a proton α to the acetoxyl. The low field position suggested probability of the presence of a vicinal glycol system. The other proton α to acetoxyl was merged with the solvent

peak at δ 4.86 (CD_3OD). In the NMR spectrum recorded in $CDCl_3$, the H-3 signal could be located at δ 4.8 as a doublet (J=10Hz) indicating diaxial relationship between H-2 and H-3 leading to 2α, 3β-glycol system.

In the NMR spectrum of the acetate, the two acetoxyls were at δ 1.96 and δ 2.05. In the methyl region there were four tertiary methyls at δ 0.93, δ 1.02, δ 1.09 and δ 1.25 and a secondary methyl as a doublet centred at δ 1.09 and a vinylic methyl at δ 1.73. There was a triplet at δ 5.36 and a multiplet at δ 5.76 each integrating for one proton corresponding to an olefinic proton Δ^{12} and a proton α to the acetoxyl H-2, H-3 signal was partly merged with the solvent signal near δ 4.8.From the foregoing data, the compound appears to be an α-amyrin based triterpene having the two hydroxyls, two carboxyls and a double bond in ring E in addition to the Δ^{12} double bond. The presence of one vinylic methyl at δ 1.73, one secondary methyl at δ 1.09 and an extra olefinic proton signal at δ 5.45 indicates that the double bond is located between C-20/C-21 position.

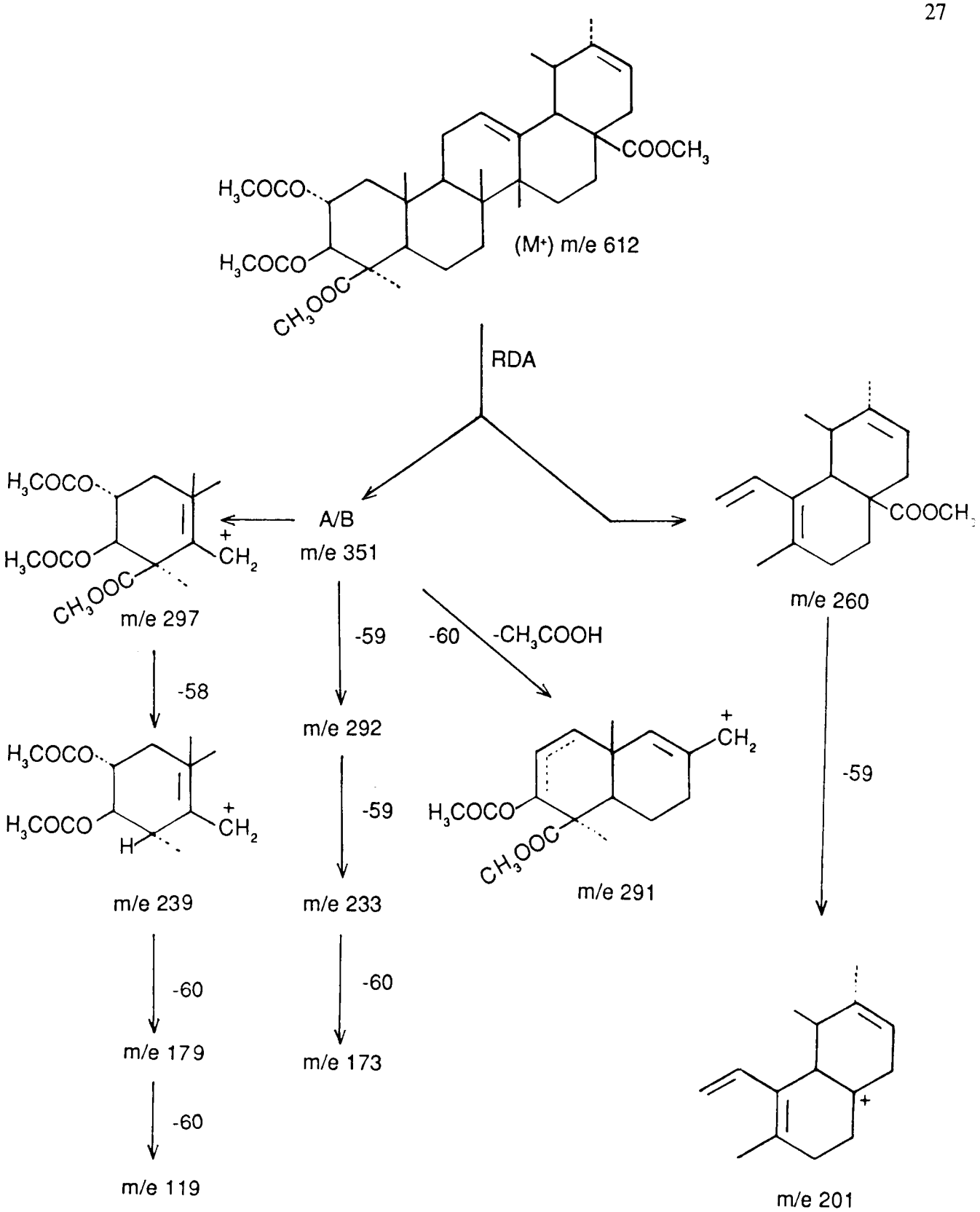
In the mass spectrum of the acetyl methyl ester the molecular ion peak was observed at m/e 612 as expected. The RDA fragment arising from D/E ring was located at m/e 260 indicating location of one -$COOCH_3$ in the D/E rings. Loss of COOMe moiety gave rise to a very intense peak at m/e 201. The A/B ring containing RDA fragment was expected at m/e 351, though this peak was not abundant, a peak at m/e 291 corresponding to loss of one acetic acid unit was more prominent. These data suggest that one carboxyl and two hydroxyl have to be located in A/B rings and one carboxyl in D/E rings. Coupled with the NMR shift for H-2, which is comparatively downfield, it suggested a vicinal glycol system. A tentative structure could be 2,3-dihydroxy-urs-$\Delta^{12,20}$-diene 23/24, 28 dioic acid. The fragmentation pattern has been shown in **Chart-III**.

LOCATION OF COOH GROUP

A study of the I.R. spectrum showed that the carboxyl in the A ring is also axial in view of the absence of strong absorption at 1245 cm^{-1}. Hence it is concluded that this could be **2α, 3β-dihydroxy-urs-$\Delta^{12,20}$-diene 24, 28 dioic acid**. As this compound is **new** and is being reported **first time**, it is therefore named as **corchorenic acid**.

The ^{13}CNMR spectrum of the sapogenin acetate (Corchorenic acid acetate) m.p 277-78^0 taken in $CDCl_3$ also leads to the same conclusion. In the low field region, there are four carbonyl

CHART-III


COOCH3
H3COCO
H3COCO
CH3OOC
(M+) m/e 612
RDA
A/B
m/e 351
m/e 297
m/e 260
-59
-60
-CH3COOH
-58
m/e 292
m/e 239
m/e 291
-59
-59
m/e 233
-60
-60
m/e 179
m/e 173
-60
m/e 119
m/e 201

signals at δ 186.5, δ 181.5, δ 171.5 and δ 171.0. Of these the last two can be assigned to the carbonyl of the two acetate moieties, while the other two can be assigned to the two carboxyl groups. In the olefinic region, there are four signals at δ 145.0, δ 138.0, δ 129.0 and δ 125.5 of which the first two appeared as singlets in the off-resonance while the last two appeared as doublets indicating that these two arise from protonated carbons. Of these, signals at δ 129.0 and δ 138.0 can be assigned to C-12 and C-13. Obviously signals at δ 125.5 and δ 145.0 arise from the carbons of the other olefinic unit which should be tri-substituted. Hence, these could be assigned to C-20(δ 145.0) and C-21(δ 125.0). Besides these there were signals in aliphatic region which could not be analysed in detail.

STUDY OF THE WATER INSOLUBLE FRACTION W-INS(ISOLATION OF DEPRESSIN, A NEW TRITERPENIC GLYCOSIDE)

The water insoluble fraction was dissolved in ethyl acetate under refluxing conditions. After concentrating the solution to a small volume and leaving in the fridge for 3 days, a solid "**B**" separated out which was filtered. The filtrate (mother liquor) was coded as **"BM".**

The solid **"B"** on acetylation at room temperature gave an acetyl derivative (Depressin acetate), m.p. 195°.

In order to isolate Depressin acetate from the drug procured from Hamdard Dawakhana, two modified procedures as per the details given in the **Experimental part** were used.

On methylation with diazomethane, the acetate gave the acetyl methyl derivative, m.p. 169-70°.

The NMR spectrum of the above acetyl methyl ester showed six methyl at δ 0.66, δ 0.85, δ 0.88, δ 1.1 and δ 1.2, the last peak integrating to two methyls. Further, the peak at δ 0.88 appeared as a doublet with J= 7Hz accounting for one secondary methyl,while all the other five being tertiary methyls. The acetoxyl region integrated to six acetoxyl groups, with the peaks located at δ 1.93(3H), δ 1.96(3H), δ 1.97(6H), δ 2.01(3H), δ 2.03(3H). Further the methoxyl region revealed a single methoxyl appearing as a singlet at δ 3.65. Taking together all the above features, the compound appears to be the glycoside of compound "AA" in which the sugar is attached through a carboxyl. The total resemblance of peaks at δ 4.8(d, J=10Hz) for H-3, a triplet

centred at δ 5.31 for Δ^{12} proton and a six line pattern centred at δ 5.65, for H-2 with the corresponding region of the spectrum of compound "AA", leads to the same conclusions as already stated above. The protons attached to the sugar moiety could be partly analysed as follows.

The multiplet centred at δ 3.7 could be assigned to H-5' as it is coupled to both H-4 and CH_2 groups(methylene hydrogens). The two double doublets centred at δ 4.0 and δ 4.2 could be assigned to the non-equivalent methylene hydrogen. The complex group of peaks centred at δ 5.15 integrating for 3 hydrogens may be due to C-2', C-3' and C-4' hydrogens. The anomeric hydrogen appeared as a sharp doublet centred at δ 5.4(J=8Hz). The last observation is more in conformity with the β-glycoside configuration, as the C-1 hydrogen will have a diaxial relationship with C-2 hydrogen. Thus this compound appears to be a monoglycoside of compound "AA". The location of the sugar unit is obviously on one of the carboxyls in view of the absence of one methyl ester signal and the presence of six acetoxyls.

HYDROLYSIS OF THE ABOVE GLYCOSIDE AND IDENTIFICATION OF SUGAR

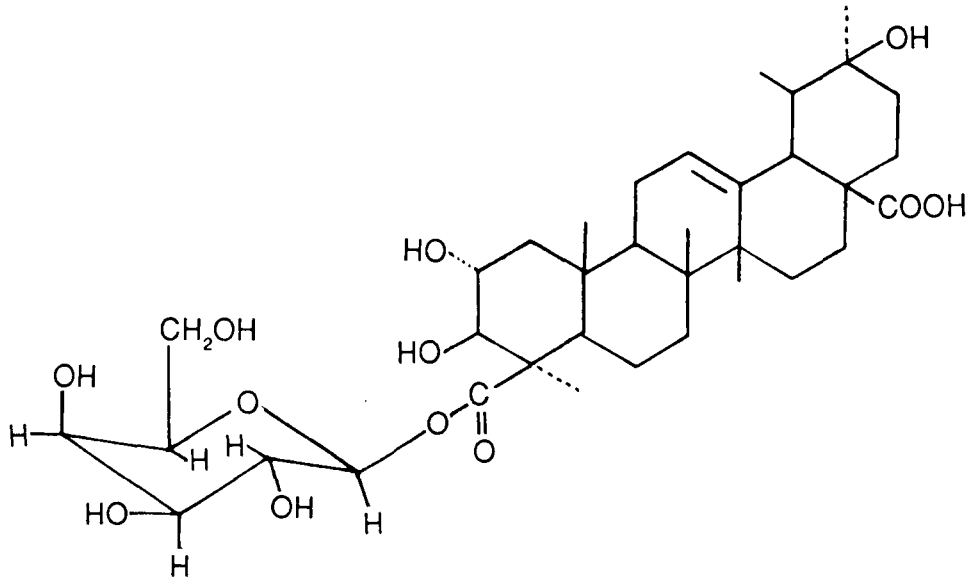
The glycoside could not be hydrolysed by heating with dil. HCl under ordinary conditions and hence it was subjected to hydrolysis by Killiani's method. The hydrolysate was extracted several times with ether in order to remove the aglycone and the aqueous layer was tested for the presence of sugar. On paper chromatographic examination of the test material alongside with different authentic samples of sugars, it was identified as galactose. The ethereal layer on usual work up gave an aglycone which was acetylated and purified by column chromatography to give the acetyl derivative m.p. 262-63^0 which was identical with "AA". In another set of experiments, depressin acetate was hydrolysed by methanolic sodium hydroxide. The aglycone obtained was identified as 2α, 3β, 20β-trihydroxy-urs-Δ^{12}-ene 24,28 dioic acid.

POSITION OF THE SUGAR LINK

The glycoside acetate methyl ester was subjected to saponification and the saponification product was reacetylated. The mass spectrum of this reacetylated product did not show the molecular ion peak, however it gave peaks at m/e 278, 260, 218, 219, 201, 146 & 133 which are present in the mass spectrum of compound "AAM". All these peaks originate on the D/E rings and hence C-17 carries -$COOCH_3$. It is not surprising that this ester moiety is not hydrolysed by alkali,

in view of the known hindrance to hydrolysis. Hence, these data lead to the location of the sugar at the other carboxyl attached at 4-position of ring-A as shown in the formula.

The same conclusions can be reached from analysis of the NMR spectrum of the above acetate which showed six C-methyls from δ 0.712 to δ 1.25. There were two acetoxyl signals at δ 1.97 and δ 2.07 and an ester methoxyl signal at δ 3.7. Besides these there was a one proton doublet at δ 4.79(J=7.7 Hz) and a one proton multiplet at δ 5.32 and another one proton unresolved signal at δ 5.69. Obviously these arise from H-3, H-12 & H-2. Since the methoxyl function is present, it indicated the carbomethoxy group is stable to saponification hence must be present at a hindered position i.e. at C-17 which is well recorded in amyrin group of triterpenes.This leads to the conclusion that the sugar is attached to the other carboxyl i.e. at 24 position.



The glycoside was therefore identified as **2α, 3β, 20β-trihydroxy-urs Δ^{12}-ene 24, 28 dioic acid-24-βD-galactoside**. As this glycoside is **new** and is being reported for the **first time**, it is therefore named as **Depressin**.

STUDY OF THE MOTHER LIQUOR (BM) LEFT AFTER FILTERING OFF SOLID "B" (ISOLATION AND CHARACTERISATION OF FLAVONOIDS FL-1 AND FL-2)

The solvent (ethyl acetate) of the mother liquor **(BM)** was evaporated off to dryness and the residue was exhaustively extracted with benzene. The benzene insoluble solid gave positive tests for flavonoids. It was chromatographed on a column of silica gel and eluted successively with petroleum ether, benzene, benzene-ethyl acetate mixture and finally with ethyl acetate.

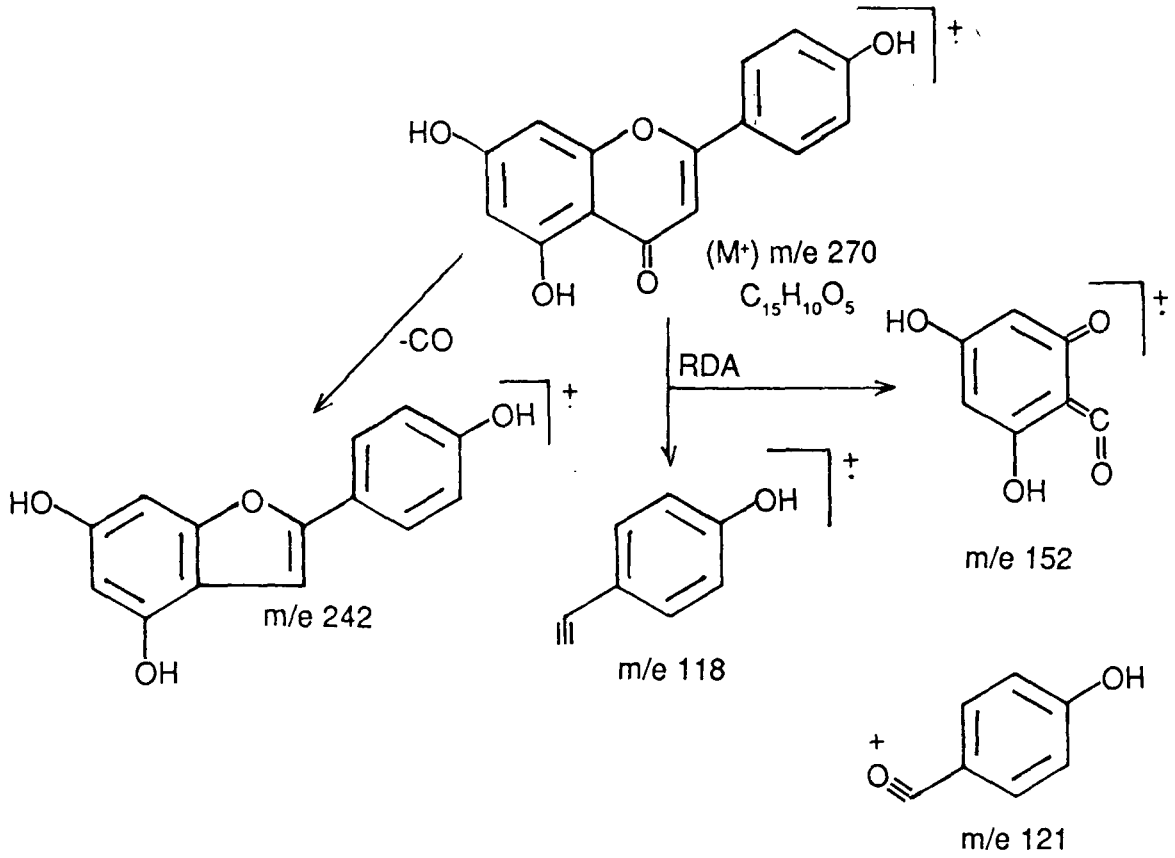
The first two fractions of (100 ml each) eluted by benzene-ethyl acetate mixture(4:1) gave a product, m.p.298° (coded as **FL-1**) which gave positive tests for flavonoids. Further elution with the same solvent mixture gave a product which on T.L.C. examination showed two brown spots under U.V. light with Rf values 0.75 and 0.64. By preparative T.L.C. this mixture was separated into two pure compounds. One of these compounds was identical with **FL-1** and was identified as **apigenin**, while the other compound (Rf 0.64) was coded as **FL-2**.

CHARACTERISATION OF FL-1

The U.V. spectrum of the compound showed two maxima at 268.8 (Band-II) and 337.6 nm (Band-I) with an inflexion around 306 nm. With $AlCl_3$ it showed four bands at 276.8, 301.6, 344.8 and 383.2 nm. The shift in band-I indicated the presence of a 5-OH group. On addition of sodium acetate, it showed three bands at 275.2, 308.4 and 385.6 nm. The shift in Band (II) was indicative of a 7-OH group. Addition of boric acid gave two bands at 268.8 and 338.8 nm with an inflexion around 300 nm. Absence of shift with this reagent in Band(I) ruled out the presence of ortho dihydroxy system in the side phenyl ring. These data are indicative of 5,7,4'-trihydroxy flavonoids with no oxygenation at 3-position . A comparison of the data with apigenin suggested that the compound may be apigenin.

These findings were further supported by the mass spectral data. The molecular ion peak was located at m/e 270, consistent with the trihydroxyflavone structure. Loss of CO led to a peak at m/e 242 and RDA cleavage gave the A-ring fragment at m/e 152 and also at m/e 153 i.e. 152+H and the B-ring fragment at m/e 118. The acylium ion fragment originating from B-ring was located at m/e 121.These data suggested that the two of the hydroxyls are located in the A-ring and one OH group is located in the B-ring. The fragmentation pattern has been shown in **Chart-IV**. The above (mass) data compared well with that reported for apigenin. Its final identity as **apigenin** was established by T.L.C. comparison of the sample with an authentic sample.

CHART-IV



CHARACTERISATION OF FL-2(Rf 0.64)

It gave positive tests for flavonoids. The U.V. spectrum of the compound in methanol showed maxima at 254.0, Band(II) 268.0 and 349.6 nm Band(I) with a shoulder at 290 nm suggesting that the compound may be a flavone. Addition of $AlCl_3$ shifted the longwave length band to 422.4 nm, thus producing a bathochromic shift of 72.0 nm suggesting the presence of a 5-OH group.Addition of HCl to the $AlCl_3$containing solution produced a hypsochromic shift indicating a catecholic unit. This is also supported by addition of boric acid to the NaOAc containing solution which produced a shift of 23.2 nm of band-I relative to the original spectrum. Finally NaOAc produced a bathochromic shift in the short wavelength band (Band II) indicating a 7-OH group. Thus the compound appeared to contain a 5,7 - dihydroxy system in the A- ring of the flavone skeleton and the ortho dihydroxy unit in the B- ring. The above U.V. data compared well with that reported for luteolin. Its final identity as **luteolin** was established by T.L.C. comparison of the sample with an authentic sample.

EXPERIMENTAL

STUDY OF THE WHOLE PLANT OF *CORCHORUS DEPRESSUS* (LINN) SYN.,*C.ANTICHORUS* (RAEUSCH) OR *ANTICHORUS DEPRESSUS* (LINN) (N.O. TILIACEAE)

EXTRACTION

The dried plant material consisting of whole plant was purchased from two places in Delhi, namely Kharibaoli Market and Hamdard Dawakhana (Wakf). The authenticity of the purchased materials was established in the Department of Botany (University of Delhi) and in the pharmacognosy section of our Institute. Both of them gave very similar results. The material purchased from Kharibaoli (3 kg) was extracted with hot ethanol eight times till the last extract became colourless. The combined extracts were concentrated to a small volume and left in refrigerator overnight. A solid product (60 gm) deposited in the flask which was filtered, washed with a small quantity of ethanol and dried. It was coded as **'A'**. The filtrate was evaporated to dryness and successively extracted with petroleum ether (60-80°), chloroform and acetone in order to give the respective extracts **E-1**, **E-2** and **E-3**.

STUDY OF PRODUCT 'A'

The product **'A'** (10.0 gm) was refluxed with petroleum ether (60- 80°) in order to remove waxy and other petrol soluble fractions.The petrol insoluble residue gave a purple violet colour in Liebermann-Burchard test. As it was found to be highly insoluble in usual solvents it was therefore converted into its acetyl derivative.

ACETYLATION

The petrol insoluble product(7.0 gm) was dissolved in hot pyridine (30 ml) and filtered. Acetic anhydride (25 ml) was added to the filtrate and the contents heated for three hours on a boiling water bath and then left overnight at room temperature. The reaction mixture was then poured into ice cold water with constant stirring, a precipitate separated out, which was filtered and washed with water in order to make it free from pyridine and acetic acid. It was allowed to dry at room temperature. It gave a purple violet colour in L.B. test for triterpenes.

PURIFICATION OF THE ACETATE

The dried crude acetate (6.1 gm) was dissolved in a large volume of methanol and charcoaled three times. The filtrate was concentrated and left at room temperature for crystallisation,when a colourless crystalline compound (m.p. 262-63°, 1200 mg) was obtained. On T.L.C. examination in toluene: ethyl formate: formic acid (5:4:1),it showed a single spot. It was coded as **'AA'** .

METHYLATION OF THE ACETATE (AA) WITH DIAZOMETHANE

The acetate (500 mg) was taken in a 250 ml conical flask and treated with an excess of ethereal solution of diazomethane. The contents were left overnight at room temperature.The ethereal solution was then evaporated to dryness. The residue so obtained was crystallised with aqueous methanol to give a colourless crystalline compoud (350 mg, m.p. 185-86°). It was coded as **'AAM'**.

SPECTRAL DATA

^{1}HNMR (δ, $CDCl_3$)

0.629(s,3H, CH_3), 0.849(s, 3H, CH_3), 0.894(d, J=5Hz, 3H, CH_3), 1.15(s, 3H, CH_3), 1.209(s, 3H, CH_3), 1.217 (s, 3H, CH_3), 1.9297(s, 3H, $OCOCH_3$), 2.0299 (s, 3H,$OCOCH_3$), 3.65(s, 3H, $COOCH_3$), 3.75(s,3H, $COOCH_3$), 4.75(d, J=10Hz, 1H, H-3), 5.288(t, J = 5Hz, 1H, H-12), 5.66(dt, 1H, H-2).

dt = doublet of triplet

MASS (m/e)

630(M^+) peak not observed, 570, 555, 510, 351, 297, 292, 278, 260, 238, 233, 219, 218, 201, 146, 133.

DEACETYLATION OF THE ACETATE (AA)

The acetate (300 mg) was refluxed with methanolic sodium hydroxide (70 ml, 4%) on a water bath for four hours and then after evaporating off half of the solvent, the reaction mixture was diluted with water (200 ml) which did not give any insoluble product, it was therefore acidified with hydrochloric acid. A colourless precipitate so obtained was filtered, washed with water till free from acid and dried. The filtrate was extracted with solvent ether in a separating funnel twice.The ethereal extracts were combined together and washed with water to remove acid. It was then dried over anhydrous sodium sulphate and after filtering off sodium sulphate, ether was distilled off. A residue in small quantity was obtained which was mixed with the above precipitate and crystallised with methanol. A colourless crystalline compound (180 mg), m.p. > 360° was obtained which was identified as 2α, 3β, 20β-trihydroxy-urs–Δ^{12}-ene-24,28 dioic acid.

SPECTRAL DATA (^{13}CNMR, DMSOd_6)

As detailed in discussion (page 20 and 22)

STUDY OF PETROL EXTRACT E-1

The petrol was distilled off from the extract to give a residue (20.0 gm).

The residue (20 gm) was chromatographed on a column (2.5 cm. in diameter) of silica gel (200 gm, 60-120 mesh) and eluted with petroleum ether (1.5 lit), petroleum ether-benzene mixture (1:1, 500 ml), benzene (500 ml), acetone(500 ml) and finally with alcohol(400 ml). The petrol-benzene (1:1) fraction (500ml) eluted from the column was concentrated to dryness. It was crystallised from methanol to give a light brown sticky compound(1200 mg, m.p.87°). On T.L.C. examination in petrol (60- 80°), toluene, ethyl acetate; 10,5,3, it was found to be a mixture of more than two components. The other eluates did not give any appreciable quantity of pure material and hence no further work was carried out.

ISOLATION OF β-SITOSTEROL

The above compound (m.p. 87°, 1200 mg) was rechromatographed on a column (1.0 cm. in diameter) of silica gel (12 gm, 60-120 mesh) and eluted with petroleum ether (60-80°, 500 ml),

petrol- benzene mixtures, (9:1 and 3:1, 500 ml each), benzene (300 ml) and finally with acetone(200 ml). The petrol-benzene(3:1) eluate (500 ml) was evaporated to dryness and crystallised from methanol to give a colourless crystalline compound (500 mg, m.p. 137°). It gave a green colour in L.B. test. By co-T.L.C with an authentic sample of β-sitosterol in petrol (60-80°), toluene, ethyl acetate; (10:5:3) it was found to be identical (m.m.p with β-sitosterol 137°). IR spectrum of the compound was superimposable with that of an authentic sample.

ACETYLATION OF THE ABOVE COMPOUND

The above compound (200 mg) was dissolved in pyridine (2.0 ml) and acetic anhydride(2 ml) was added to it. It was heated on a boiling water bath for two hours and left at room temperature overnight. It was then poured into ice cold water to give a colourless precipitate which was filtered, washed with water to make it free from acetic acid and pyridine. On crystallisation from methanol, it gave a colourless compound, m.p. 126° (175 mg). By co-T.L.C. in petrol (60-80°), toluene, ethyl acetate (10:5:3) alongwith an authentic sample of β-sitosteryl acetate, it was found to be identical. It did not show any depression in m.p. on admixture with an authentic sample of β-sitosteryl acetate (m.m.p. 126°).Its IR spectrum was superimposable with the I.R. spectrum of β-sitosteryl acetate.

STUDY OF CHLOROFORM EXTRACT (E-2)

The chloroform extract was evaporated to dryness to give a residue which gave a brownish red colour in L.B. test.

SEPARATION OF CHLOROFORM CONCENTRATE INTO NEUTRAL AND ACIDIC FRACTIONS

The above residue (5 gm) was stirred with an aqueous sodium hydroxide solution (4%, 200 ml) and then heated on a boiling water bath for one hour. After cooling to room temperature, it was left in the refrigerator overnight and then filtered. The residue (1.0 gm) was washed with water to make it free from alkali. It was labelled as neutral fraction **(F-1)** . The filtrate was acidified with HCl to give a brown precipitate which was filtered and washed with water till free from acid. It was labelled as acidic fraction (**F-2**, 3.5 gm).

STUDY OF THE NEUTRAL FRACTION (F-1), ISOLATION AND CHARACTERISATION OF β-SITOSTEROL GLUCOSIDE AS ITS ACETATE

The neutral fraction was dissolved in pyridine(3.0 ml) and acetic anhydride(2.0 ml) was added to it. The contents after heating on a boiling water bath for two hours were left at room temperature for 24 hours and then worked up as usual to give the crude acetyl derivative which crystallised from methanol containing a few drops of chloroform to give a colourless compound (250 mg, m.p. 166°). It gave a green colour in L.B. test. On. T.L.C. examination in toluene, ethyl acetate(8:2),it was found to be a single entity.

SPECTRAL DATA

^{1}HNMR (δ, CD_3OD)

0.717-1.012(6xCH_3), 1.956(s, 3H, $OCOCH_3$), 1.999(s,3H, $OCOCH_3$), 2.020(s,3H, $OCOCH_3$), 2.044(s,3H, $OCOCH_3$), 4.2-5.1(m, 7H, protons α to acetoxyl), 5.4(m,1H, H-5).

MASS(m/e)

(M^+) Peak not observed, 396,255, 214.

DEACETYLATION OF THE ABOVE GLYCOSIDE ACETATE

The acetate (100mg) was refluxed with methanolic sodium hydroxide (25ml, 4%) on a boiling water bath for four hours and then processed as usual to give the crude deacetylated product which crystallised from methanol to give a colourless crystalline compound (50 mg, m.p. 282°). On T.L.C. examination in benzene,methanol; (9:1) it showed a single spot.

KILLIANI'S HYDROLYSIS OF THE ABOVE DEACETYLATED PRODUCT

The compound (25 mg) was taken in a thick walled test tube and 2.0 ml of Killiani's mixture (glacial acetic acid: conc. hydrochloric acid: water; 7:2:11) was added to it. The contents were heated on a boiling water bath for three hours after tightly fixing a cork on the mouth of the test tube and tightening it further with the help of a thread. The reaction mixture on dilution with water gave

a precipitate which was extracted with solvent ether three times (3x25 ml) in a separating funnel. All the ethereal extracts were combined together and washed with water to remove acid. The ethereal extract was then dried over anhydrous sodium sulphate and filtered to remove inorganic salts. Recovery of ether gave a residue which on crystallisation with methanol gave a colourless compound (5.0 mg, m.p.137°). By co-T.L.C. petrol(60-80°): toluene: ethyl acetate, (10:5:3) with an authentic sample of β-sitosterol, it was found to be identical with it. Melting point of the compound when mixed melted with an authentic sample of β-sitosterol did not show any depression (m.m.p. 137°).

IDENTIFICATION OF THE SUGAR

The aqueous layer left after extraction with ether was evaporated to dryness in a china dish on a water bath. The residue was dissolved in water (2 ml). It gave a red precipitate by heating with Fehling's solution indicating the presence of reducing sugar. The aqueous solution was applied on Whatmann filter paper no. 1 and chromatographed by descending technique alongside with authentic samples of different sugars. The solvent system used was n-butanol:ethanol: water(4:1.1:0.9). The chromatogram was allowed to run at room temperature for 30 hours. It was then removed from the chamber, dried and after spraying with aniline hydrogen phthalate, left in an oven at 105° for ten minutes. The developed spot of the test material corresponded well with the sugar glucose and hence the sugar was identified as glucose.

STUDY OF ACIDIC FRACTION(F-2),ISOLATION OF COMPOUND 'AA'

This fraction gave a pink colour by L.B.test for triterpenes.

ACETYLATION

The above fraction **F-2** (3.5 gm) was dissolved in dry pyridine (5.0 ml) and acetic anhydride(5.0 ml) was added to it. The contents were heated on a boiling water bath for four hours and left overnight at room temperature. On usual work up,it gave a crude acetate which after charcoaling and crystallisation with methanol gave a crystalline compound m.p. 260°. It agreed with "AA". On methylation with diazomethane, it gave dimethyl ester diacetate, m.p. 185-86° (AAM).

STUDY OF ACETONE EXTRACT(E-3), SEPARATION INTO WATER SOLUBLE (W-S) AND WATER INSOLUBLE (W-INS) FRACTIONS.

The acetone concentrate (100 gm) was taken up in water (2 lit.) and filtered to give a water soluble (**W-S**) and water insoluble fraction (**W-INS**) (40.0 gm).

HYDROLYSIS OF THE WATER SOLUBLE FRACTION (W-S), ISOLATION AND CHARACTERISATION OF A NEW TRITERPENIC ACID NAMED AS CORCHORENIC ACID

This fraction (**W-S**) gave a positive test (stable copious foams on shaking with water) for the presence of saponins.

It was hydrolysed by dil. H_2SO_4 (4%, v/v) by heating first on a boiling water bath for four hours and then by refluxing for another one hour on a wire gauze. A dark brown aglycone(6.0 gm) separated out which was filtered and washed with water several times to make it free of acid. It gave a positive L.B. test (red colour) for triterpenoids.

ACETYLATION

The above aglycone (6.0 gm) was dissolved in pyridine (12.0 ml), acetic anhydride (10.0 ml) was added to it. The reaction mixture was heated on a boiling water bath under anhydrous conditions for four hours and left at room temperature overnight. After processing in the usual way, a crude product was obtained which on crystallisation with methanol (charcoal) gave a colourless compound (260-63°, 400 mg). On T.L.C. examination in toluene: ethyl formate: formic acid (5:4:1) it showed only one elongated spot indicating its impure nature.

DEACETYLATION

The above acetate (350mg) was refluxed with methanolic sodium hydroxide (70ml, 4%) on a boiling water bath for four hours. After removing the condenser, half of the solvent was evaporated off and the rest of the reaction mixture was diluted with water (200 ml). As no insoluble product separated out, it was therefore acidified with hydrochloric acid to give a colourless precipitate which was filtered, washed with water and dried. The filtrate was extracted with solvent ether three times. The ethereal extracts were combined together, washed with water, dried over

anhydrous sodium sulphate and after filtering off inorganic salts evaporated to dryness to remove the solvent. This process gave a small residue which after T.L.C. examination,was mixed with the above precipitate. On crystallisation with methanol, it gave a colourless hexagonal crystalline compound(m.p. 295°, 200 mg) which was T.L. C. pure in toluene : ethyl formate : formic acid; (5:4:1).

ACETYLATION OF THE ABOVE DEACETYLATED PRODUCT

The above deacetylated product (175 mg) was dissolved in pyridine (1.5 ml) and acetic anhydride(1.0 ml) was added to it. The contents were heated on a boiling water bath for two hours and left at room temperature for 24 hours. The reaction mixture on usual work up and crystallisation with methanol gave a colourless compound (m.p. 277-78°, 150 mg).

SPECTRAL DATA

^{1}HNMR (δ,CD_3OD)

0.93(s,3H, CH_3), 1.02 (s,3H, CH_3),1.09 (s,3H, CH_3), 1.09(d,J=5Hz, 3H, CH_3), 1.25 (s,3H, CH_3),1.73 (s,3H, vinyl CH_3), 1.96 (s, 3H, OAc), 2.05 (s,3H, OAc), 4.8(H-3), 5.36 (t, 1H, H-12), 5.45(m, 1H, H-21), 5.76(m, 1H, H-2)

^{13}CNMR (δ, $CDCl_3$)

As detailed in discussion (page 26 and 28)

METHYLATION OF THE ABOVE ACETATE m.p. 277-78°

The above acetate(35 mg) was treated with an excess of ethereal solution of diazomethane. The contents were left at room temperature overnight. Ether was then distilled off, and the residue crystallised with methanol to give a colourless crystalline compound(m.p. 238°, 25 mg).

SPECTRAL DATA

^{1}HNMR (δ, $CDCl_3$)

0.87(s,3H, CH_3), 0.96 (s,3H, CH_3),1.02 (s,3H, CH_3),1.09(d,J=5Hz, 3H, CH_3), 1.25 (s,3H, CH_3),1.73 (s,3H, vinyl methyl), 1.97 (s, 3H, OAc),2.04 (s,3H, OAc), 3.59(s, 3H, $COOCH_3$), 3.67 (s, 3H, $COOCH_3$), 4.8 (d, J=10Hz, 1H, H-3), 5.36 (t, 1H, H-12), 5.75(m,1H, H-2).

MASS (m/e) - As detailed in discussion. (page 26)

STUDY OF WATER INSOLUBLE FRACTION (W-INS),ISOLATION OF DEPRESSIN, A NEW TRITERPENIC GLYCOSIDE

The water insolube fraction **W-INS** (6.0 gm) was dissolved in ethyl acetate (300 ml) by refluxing on a boiling water bath for half an hour. The ethyl acetate solution was concentrated to a small volume (75 ml) and left in a refrigerator for 3 days.A solid separated out, which was filtered and washed with a small quantity of ethyl acetate. It was coded as **"B"**. The filtrate (mother liquor) was coded as **(BM)** .

The above crude product (4.5 gm) **"B"** was dissolved in pyridine (20 ml) and acetic anhydride (15 ml) was added to it. The contents after leaving at room temperature for 24 hours were worked up as usual to give the crude acetyl derivative which was crystallised from methanol (charcoaling was done three times) to give a colourless crystalline compound(m.p. 195°, 800 mg). It was observed that the above procedure when applied to isolate **DEPRESSIN** from the drug obtained from Hamdard Dawakhana did not succeed and hence the following modified methods were adopted.

FIRST METHOD

The crude acetyl derivative(1.5 gm) was subjected to column chromatography on silica gel(60-120 mesh, 75 gm, diameter of the column 2.5 cm.). The column was successively eluted with petroleum ether (300 ml), benzene (300 ml) and finally with benzene-ethyl acetate mixture (8:2, 600 ml). The benzene-ethyl acetate eluate gave a T.L.C. pure product which was crystallised with methanol to give a colourless compound m.p. 195°. It was identical with the acetyl derivative mentioned above.

SECOND METHOD

The crude acetyl derivative (2.0 gm), after dissolving in chloroform (5.0 ml) was exhaustively extracted with petroleum ether (95.0 ml) under refluxing conditions. The petrol extract was filtered. The residue left after petrol extraction was redissolved in chloroform and re-extracted with petroleum ether and filtered. This procedure was repeated third time also.All the petroleum ether extracts were combined together and evaporated to dryness. A residue so obtained was crystallised with methanol to give a colourless crystalline compound m.p. 195°. It was identical with the acetyl derivative mentioned above.

METHYLATION OF DEPRESSIN ACETATE

The acetate (450 mg) was methylated with an excess of ethereal solution of diazomethane. The contents were left overnight at room temperature. Ether was then evaporated off to dryness and the residue crystallised with aqueous methanol to give colourless fine needles (m.p. 169-70° 300 mg).

SPECTRAL DATA

^{1}HNMR (δ, $CDCl_3$)

0.66 (s,3H, CH_3), 0.85 (s,3H, CH_3), 0.88(d,J=7Hz,3H,CH_3),1.1(s,3H,CH_3), 1.2(s,6H,2xCH_3), 1.93(s,3H,OAc), 1.96(s,3H,OAc), 1.97(s,6H,2xOAc), 2.01(s,3H,OAc), 2.03(s,3H,OAc) 3.65(s,3H,$COOCH_3$), 3.7(m, 1H, H-5), 4.0(dd, H-6′a), 4.2(dd H-6′e), 4.8(d,J=10 Hz,H-3), 5.15(m,3H,H-2′,3′,4′), 5.31(t, H-12), 5.4(d,J=8Hz, H-1′), 5.65(dt, H-2).

dd = double doublet, dt = doublet of triplet

ALKALINE HYDROLYSIS OF THE ACETYL METHYL ESTER OF DEPRESSIN

The above acetyl methyl ester derivative (200 mg) was refluxed with methanolic sodium hydroxide(4%, 150 ml) on a boiling water bath for eight hours. The condenser was then removed and

the solvent allowed to evaporate off to one third of its original volume. The reaction mixture after cooling to room temperature was diluted with water (150 ml) and acidified with HCl. It gave a colourless precipitate which was filtered, washed with water and dried (100 mg).

ACETYLATION

The above hydrolysed product (100 mg) was dissolved in pyridine(1.5 ml) and acetic anhydride (1.0 ml) was added to it. The reaction mixture was heated on a boiling water bath for three hours and then after cooling to room temperature, poured dropwise into ice cold water with constant stirring. A colourless precipitate separated out which was filtered, washed with water and dried. It was crystallised with methanol to give a colourless compound (m.p. 150°, 35 mg).

SPECTRAL DATA

^{1}HNMR (δ, $CDCl_3$)

0.712-1.25(18H, 6xCH_3), 1.97(s,3H, $OCOCH_3$), 2.07(s,3H, $OCOCH_3$), 3.7(s,3H, $COOCH_3$), 4.79(d, J=7.7 Hz, H-3), 5.32(m, 1H, H-12), 5.69(m, 1H, H-2).

MASS (m/e)

As detailed in discussion (page 29)

HYDROLYSIS OF DEPRESSIN ACETATE BY KILLIANI'S MIXTURE

As this compound could not be hydrolysed by 4% HCl under normal condition, hence the hydrolysis was done by Killiani mixture(glacial acetic acid, con. HCl, water: 7,2,11).

The compound (100 mg) was taken in a test tube, Killiani's mixture (10.0 ml) was added to it. The contents were then heated by immersing the test tube in a boiling water bath for two hours after tightly fixing a cork on the mouth of test tube,and then the reaction mixture was diluted with water (60 ml). A precipitate so obtained,was filtered and washed with water. The filtrate was extracted with solvent ether (3x100 ml). All the ethereal extracts were combined together, washed with water and dried over anhydrous sodium sulphate. The ethereal solution was then filtered to

remove sodium sulphate. Ether was evaporated off to dryness to give a small residue which was added to the above precipitate after T.L.C. comparison. The hydrolysed product could not be purified through crystallisation and was therefore converted into its acetyl derivative.

PREPARATION OF ACETATE

The above compound (75 mg) was dissolved in pyridine (1.0 ml) and acetic anhydride (1.0 ml) was added to it. The contents were heated for three hours on a boiling water bath and then worked up as usual to give a light brown precipitate which was filtered, washed with water and crystallised from methanol after charcoaling. However,it could not be crystallised.

PURIFICATION OF THE ABOVE ACETATE

The above crude acetate(60 mg) was subjected to column chromatography on silica gel (60-120 mesh, 3.0 gm, diameter of the column, 1 cm). The column was successively eluted with petroleum ether(100 ml), benzene(100 ml), benzene - ethyl acetate mixture(85:15, 200 ml).The fraction eluted with benzene, ethyl acetate(85:15, 200 ml) yielded a pure compound which was crystallised with methanol to give a colourless compound (m.p. 262-63°, 20 mg). By co-T.L.C. alongwith compound "AA", it was found to be identical. No depression in m.p. was observed on mixed melting with compound "AA" (m.m.p. 262-263°). The I.R. spectra of the two compounds were also superimposable.

IDENTIFICATION OF THE SUGAR

The aqueous layer left after extraction with ether (Killiani's hydrolysis of depressin acetate) was processed for the identification of sugar as described on page 38. The developed spot of the test material corresponded well with the sugar,galactose and hence,the sugar was identified as galactose.

ALKALINE HYDROLYSIS OF DEPRESSIN ACETATE

The acetate(100 mg) was refluxed with methanolic sodium hydroxide (100 ml, 4%) on a boiling water bath for eight hours. The condenser was then removed and the solvent allowed to evaporate off to half its volume. The reaction mixture,after cooling to room temperature,was diluted

with water (200 ml) which did not give any insoluble residue. On acidification with HCl, it gave a colourless precipitate which was filtered and washed with water. The filtrate was shaken with solvent ether three times (3x100 ml). All the ethereal extracts were combined together and washed with water in a separating funnel by shaking. The ethereal layer was removed and dried over anhydrous sodium sulphate. After filtering off sodium sulphate, ether was recovered which left a small residue. It was combined with the above precipitate after T.L.C. comparison. The combined precipitate was crystallised with methanol. A colourless compound(m.p. 360°, 50 mg) was obtained. By co-T.L.C. alongwith 2α, 3β, 20β-trihydroxy-urs-Δ^{12}-ene-24, 28 dioic acid (**AA**), it was found to be indetical.The I.R. spectra of the two samples were also superimposable.

STUDY OF THE MOTHER LIQUOR (BM) AFTER FILTERING OFF SOLID"B"

The mother liquor (**BM**) evaporated to dryness to give a solid (1.2 gm) which was exhaustively extracted with benzene several times. The benzene insoluble residue(1.0 gm) gave a pink colour with Zn/HCl indicating its flavonoidal nature. It was chromatographed on a column of silica gel (60-120 mesh, 30 gm, column width 2.5 cm). The column was eluted with petroleum ether (100 ml), benzene (100 ml), benzene-ethyl acetate(4:1, 7x100 ml and 1:1, 500 ml) and finally with ethyl acetate (300 ml).

ISOLATION OF FL-1

The first two fractions of 100 ml (ethyl acetate-benzene, 1:4) showed a single brown spot on T.L.C. examination under U.V. light. These two fractions were combined together and evaporated to dryness, the residue was crystallised with methanol to give a yellow crystalline compound (m.p.298°, 10 mg) It gave a pink colour with Zn- HCl, an intense yellow colour on exposure to ammonia vapours, a reddish brown colour with alcoholic $FeCl_3$ solution and a brown flourescence under U.V. light. By T.L.C. comparison with apigenin, it was found to be identical.

SPECTRAL DATA

U.V. (MeOH)

λ_{max}^{MeOH},268.8, 306(inf), 337.6 nm.

$\lambda^{AlCl_3}_{max}$,276.8, 301.6, 344.8, 383.2 nm.

$\lambda^{AlCl_3 + HCl}_{max}$,277.2, 300.4, 341.2, 376.4 nm.

λ^{NaOAc}_{max},275.2, 308.4, 385.6 nm.

$\lambda^{NaOAc+Boric\ acid}_{max}$,268.8, 300.0(inf), 338.8 nm.

MASS (m/e)

270(M^+), 242, 153, 152, 121, 118.

Due to poor yield of the compound,its acetyl derivative could not be prepared.

ISOLATION OF FL-2

Further elution of the column with benzene-ethyl acetate mixture(4:1) 500 ml, showed two brown spots (Rf 0.75 & 0.64) under U.V. light. This mixture was separated by preparative T.L.C. on silica gel-G using (benzene:ethyl acetate:formic acid; 5:3:0.4) as solvent system. 25 plates (size 20 cmx20 cm.) were run. The two bands were carefully scrapped from the plates and transferred into two separate small columns. On elution with methanol and evaporation of the solvent, two fractions were obtained. The fraction from the upper band was found to be identical with apigenin. The fraction from the lower band gave a pink colour with Zn-HCl, a green colour with $FeCl_3$ and an intense yellow colour on exposure to ammonia vapours. It gave a brown flourescence under u.v. light. By co-T.L.C. alongwith an authentic sample of luteolin in the solvent system,benzene: ethyl acetate and formic acid (5:3:0.4), it was found to be identical with it.

SPECTRAL DATA

U.V. (MeOH)

λ_{max}^{MeOH},254.0, 268.0, 290(inf), 349.6 nm.

$\lambda_{max}^{AlCl_3}$,273.6, 332.0, (inf), 422.4 nm.

$\lambda_{max}^{AlCl_3 + HCl}$,261.2,276.0(inf),295.2(inf), 358.0, 384.4 nm.

λ_{max}^{NaOAc},270.0, 324.0, 393.0 nm.

$\lambda_{max}^{NaOAc+Boric\ acid}$,262.4, 372.8, nm.

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CHAPTER - II

DISCUSSION

STUDY OF THE LEAVES OF *ICHNOCARPUS FRUTESCENS* R.Br. SYN., *APOCYNUM FRUTESCENS* LINN (N.O. APOCYNACEAE)

VERNACULAR NAMES

Hindi: Kali dudhi, Dudhilata, Siamlata, **English:** Black creeper, **Sanskrit:** Syamlata, Sariva, Paravalli, **Bengal:** Shyamlata, Dudhi, **Malyalam:** Palvali, **Marathi:** Kantebhouri, Krishnasarwa, **Tamil:** Udarkodi, Illu-Katta, **Kannad:** Kare-hambu. **Telgu:** Illukkatte, Nellatiga, **Local Name** in Bilari (Moradabad) - Boan.

DISTRIBUTION

A climbing plant which is found almost in all parts of India, ascending to an altitude of 1,200 m.[1,2]

BOTANICAL DESCRIPTION

It is an evergreen, extensively climbing and much branched shrub. Young branches, inflorescences and petioles are rusty-villous. Leaves are variable, elliptic oblong to broadly lanceolate. Flowers are greenish-white, somewhat fragrant and about 5x4 mm.in size and are found in winter. Corolla is two times long as the calyx, tube swollen round the included anthers, lobes twisted acuminate[1,2].

MEDICINAL PROPERTIES AND USES

Roots are used as demulcent, alterative, tonic, diuretic, diaphoretic and as a substitute for Indian Sarsaparilla (*Hemidesmus indicus*). Leaves and stalks are used in the form of a decoction in fever[1]. The roots are useful in skin diseases[3]. These, if tied around the neck are said to induce sound sleep[4]. The plant is considered to be useful by the tribals (Santals) in night blindness, bleeding gums, ulcerated tongue, sores, enlargement of spleen, atrophy, cachexia, convulsions, delirium, measles, small pox, haematuria, dysentery, cough, phthisis, dog bite, snake bite and spider-lick.[5]

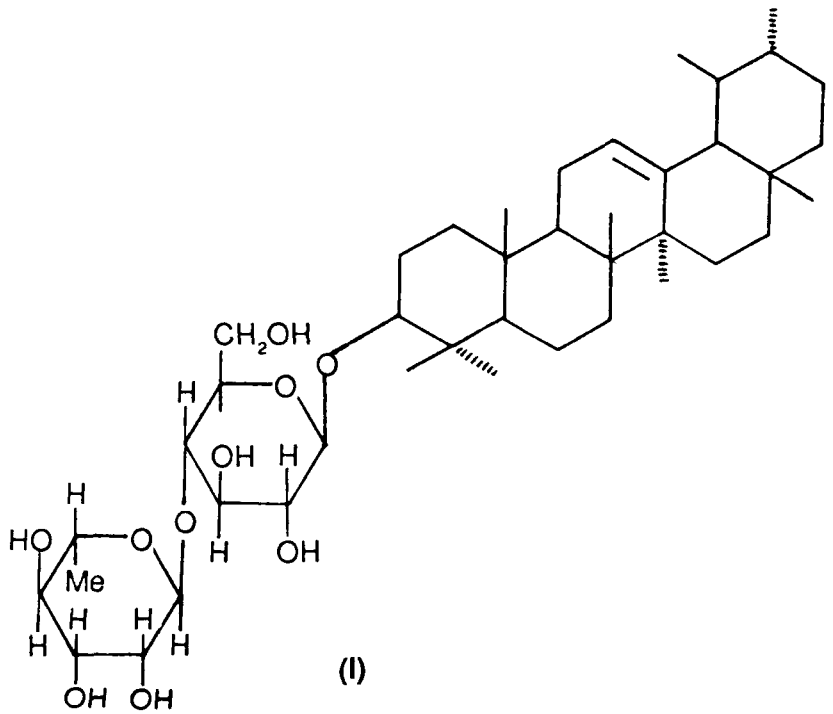
LITERATURE SURVEY

The 50% alcoholic extract of the whole plant has been reported to have antiviral activity against Ranikhet disease virus but was inactive against vaccinia virus.[6] The extract was found to be devoid of antibacterial, antifungal, antiprotozoal, anthelmintic, hypoglycaemic, anticancer activities and effect on smooth muscle preparations. The extract did not produce hypothermia and gross behavioural effects in mice. It had no effect on respiration and blood pressure in cat/dog. The maximum tolerated oral dose of the extract in mice was found to be 1000 mg/kg[6].

A survey of the literature showed that different parts of this plant have been chemically examined by different workers.

The roots have been reported to have ß-sitosterol[7] and the leaves contain apigenin, luteolin, vanilic acid, syringic acid, protocatechuic acid and sinapic acid[8]. The ethyl acetate fraction of the fresh flowers is reported to have quercetin and quercetin-3-ß-D-glucopyranoside[9].

Minocha and Tandon[10] reported the isolation of a new triterpenic glycoside from its stems which was characterised as α-L-rhamnopyranosyl-(1→4)-ß-D-glucopyranosyl-(1→3)-α-amyrin(I).



(I)

The petroleum ether extract of the stems contained α-amyrin, α-amyrin acetate, lupeol, lupeol acetate, friedilin, epi-friedinol and β-sitosterol[11]. The presence of another compound 6,8,8-trimethyl-pentacosan-7-one has also been reported from its stems[12].

Verma *etal* reported[13] that the petroleum ether extract of this plant contained Δ^{12}-dehydrolupanyl-3-ß-palmitate, lupeol acetate, friedelin, friedelinol, Δ^{12}-dehydrolupeol, oleanolic acid, nonane, 5-hydroxyoctacosan-25-one, dotriacontanoic acid, sitosterol and sitosterol-palmitate which were characterised on the basis of chemical and spectroscopic evidences. Occurrence of Δ^{12}-dehydrolupanyl 3-ß-palmitate and 5-hydroxy-octacosan-25-one were reported for the first time in nature.

As this plant is quite interesting and considered valuable in indigenous medicine, it was considered worthwhile to re-examine it, and hence these studies were initiated.

PRESENT WORK

Its fresh leaves were collected from Sanai, a neighbouring village of Tehsil Bilari in Distt. Moradabad (U.P.). The botanical identity of the plant was established at the Department of Botany, University of Delhi, Delhi-7. The fresh leaves were dried under shade and exhaustively extracted by refluxing with ethyl alcohol. The ethanolic concentrate was extracted with petroleum ether several times. All the petrol extracts were combined together and evaporated to dryness. The petrol soluble fraction was coded as **F-1**. The petrol insoluble mass was taken up in water and separated into water insoluble fraction **(F-2)** and water soluble fraction **(F-3)**.

ISOLATION OF KIL-1 FROM FRACTION F-1

The fraction **F-1** after redissolving in petroleum ether and concentrating to a small volume was left in a refrigerator overnight. A green solid separated out, was filtered. It gave a positive L.B. test and on T.L.C. examination showed two spots (one major and one minor). It could not be purified by crystallisation and was therefore acetylated. In spite of several efforts the acetate could not be crystallised from methanol. The methanolic solution of the acetate was therefore evaporated to dryness and the residue dissolved in ethanol and extracted with boiling petrol. The petrol extract on evaporation of the solvent left a semi solid mass which on crystallisation with methanol gave a colourless T.L.C. pure compound, m.p.292° coded as **(KIL-1)**. It gave a positive L.B. test

for triterpenes and was soluble in alkali showing its acidic nature. On deacetylation with methanolic NaOH and usual work up it gave the parent compound, m.p.286-87°.

The NMR spectrum of the acetate showed seven methyl functions in the range δ 0.7-δ 1.0 and one acetoxyl function as a singlet at δ 2.0. In addition to the above signals there was a multiplet centred at δ 4.46 for a proton α to the acetoxyl and a signal at δ 5.26 characteristic of the olefinic protons.

On methylation with diazomethane, the acetate gave an acetyl methyl ester (**KIL-1AM**) m.p.210°. Its NMR spectrum showed seven methyl functions between δ 0.72 to δ 1.02, a singlet for one acetoxyl functions at δ 2.0 and a singlet for the ester methoxyl function at δ 3.6.

The spectral data mentioned above for the acetate and acetyl methyl ester showed that the parent compound is a monohydroxy monocarboxylic acid triterpene. This was further confirmed by a study of the mass spectral data.

The mass spectrum of the acetate and its methyl ester showed the M^+ peaks at m/e 498 and 512 respectively. In the triterpenes, Δ^{12} compounds undergo predominantly retro-Diels-Alder fragmentation.

Thus the peaks at m/e 249 and m/e 248 in the acetyl derivative (KIL-1) are due to the fragments (I) and (II), the fragment (I) arising from rings A/B and the fragment (II) arising from D/E rings. The **charts (A)** and **(B)** showed the fragmentation pattern of the acetyl derivative (KIL-1) and its acetyl methyl ester (KIL-1AM) respectively.

A final identity of the parent compound as **ursolic acid** was established by direct comparison. On T.L.C. examination in the solvent system, petroleum ether, ethyl formate, formic acid (93:7:0.7) which is specific for distinction between ursolic acid and oleanolic acid[14], alongwith authentic samples of ursolic acid and oleanolic acid, it was found to be identical with ursolic acid (Rf 0.19).

STUDY OF WATER INSOLUBLE FRACTION F-2

The fraction **F-2** was extracted first with chloroform and then with solvent ether. Processing of the chloroform extract, followed by acetylation and purification gave a compound, m.p.290-91°, which was identified as ursolic acid acetate.

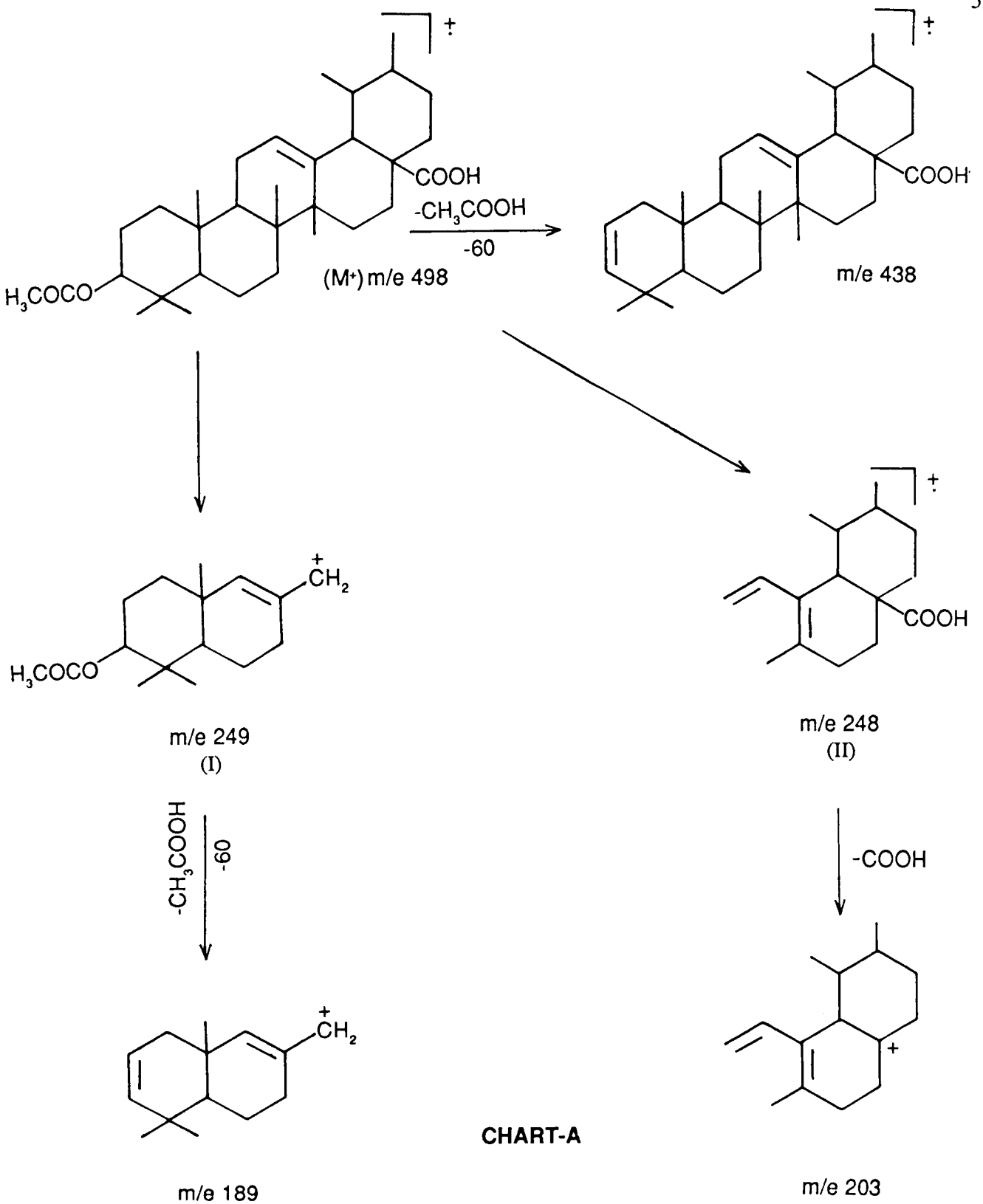

COOH
-CH3COOH
-60
H3COCO
(M+) m/e 498
m/e 438
CH2
m/e 249
(I)
m/e 248
(II)
-CH3COOH
-60
-COOH
m/e 189
m/e 203

CHART-A

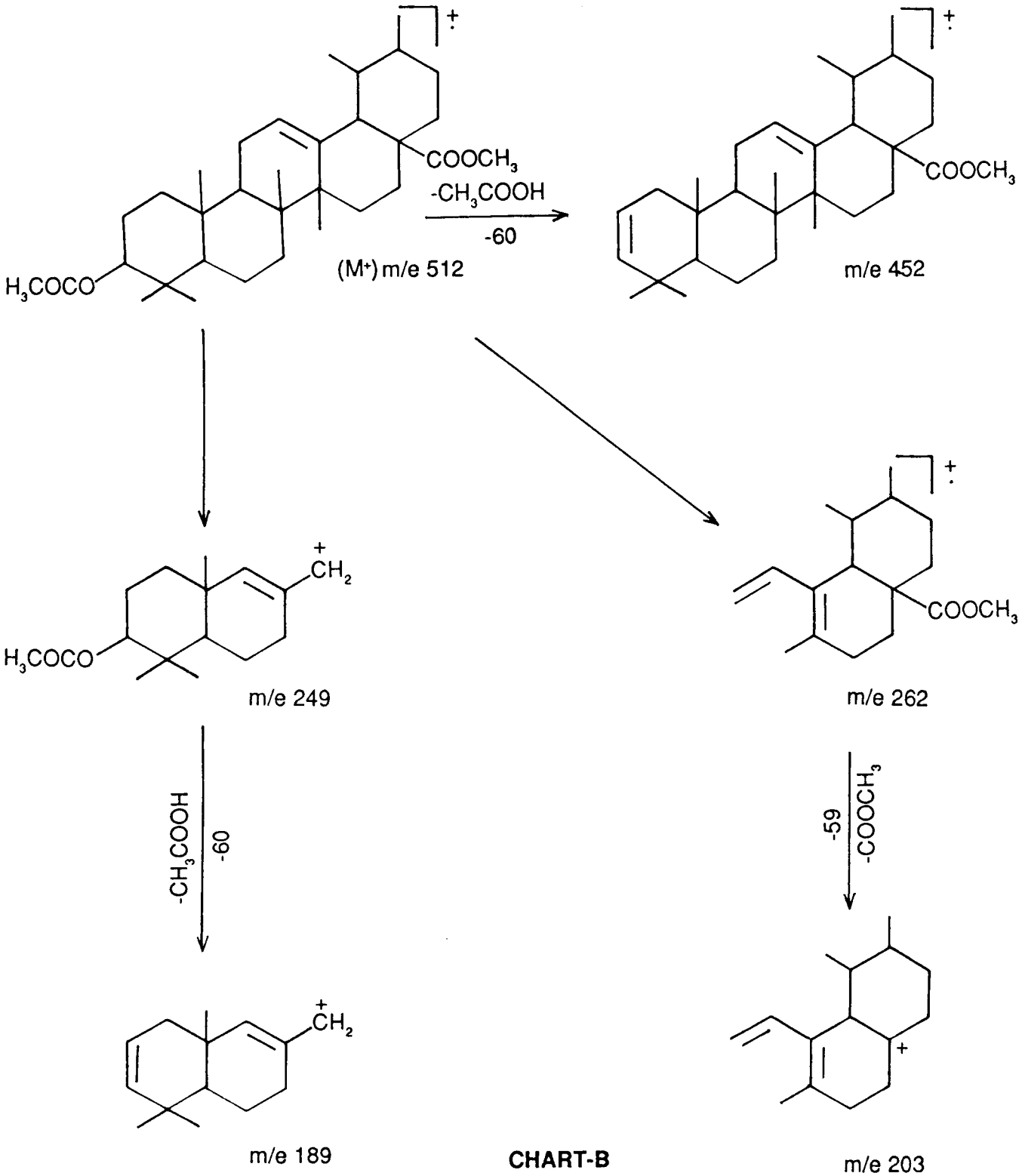

COOCH3
-CH3COOH
-60
H3COCO
(M+) m/e 512
m/e 452
CH2
m/e 249
m/e 262
-CH3COOH
-60
-59
-COOCH3
m/e 189
m/e 203

CHART-B

ISOLATION OF KIL-2

The ether extract from **F-2** on recovery of the solvent gave a brown solid which gave usual tests for the presence of flavonoids. It was purified by chromatography on a column of silica gel. The column was successively eluted with petrol, benzene, benzene-ethyl acetate mixture and finally with ethyl acetate. The first two fractions of 100 ml of benzene-ethyl acetate(9:1) eluate showed a single spot on T.L.C. examination and gave a yellow crystalline compound, m.p.246-7° coded as **KIL-2**. It gave positive tests for flavonoids.

The U.V. spectrum of the compound showed two maxima at 268 nm. and 368 nm. with two inflexions at 294 and 322 nm. Addition of $AlCl_3$ produced a very large bathochromic shift of band I to 421 nm. which was not affected by the addition of HCl. This indicated hydroxyls at 3 and 5 positions. Sodium acetate produced a bathochromic shift in band II of 7 nm. (at 275 nm.) indicating presence of 7-OH group. There was no appreciable shift in band I on addition of $NaOAc/H_3BO_3$ indicating absence of catechol. These data can be rationalised in terms of kaempferol structure for the compound.

A further support to the above structure was obtained by the mass spectral data of the compound. The mass spectrum showed the molecular ion peak at m/e 286. The other important peaks were at m/e 258, 153, 134 and 121. The fragmentation pattern has been shown below in **Chart 'C'.**

The above compound KIL-2 on treatment with acetic anhydride and pyridine gave the acetyl derivative, m.p. 118° **(KIL-2A)**. The NMR spectrum of the acetate showed four acetoxyl functions, three of them as a singlet at δ 2.3 and the fourth one as a separate singlet at δ 2.4. There was a meta-coupled doublet characteristic of H-6 at δ 6.8 (J= 2Hz). At δ 7.7 (J = 9 Hz), there was another doublet for two protons characteristic of H-2',6' . The other doublet of the A_2B_2 system was located at δ 7.3 overlapping with the signal from H-8. These data reconfirmed that the parent compound is **kaempferol**.

ISOLATION AND CHARACTERISATION OF KIL-3 FROM WATER SOLUBLE FRACTION F-3

The water soluble fraction was subjected to liquid-liquid extraction in a liquid-liquid extractor with petrol, ether, ethyl acetate and butanol successively. The petrol and ether extracts

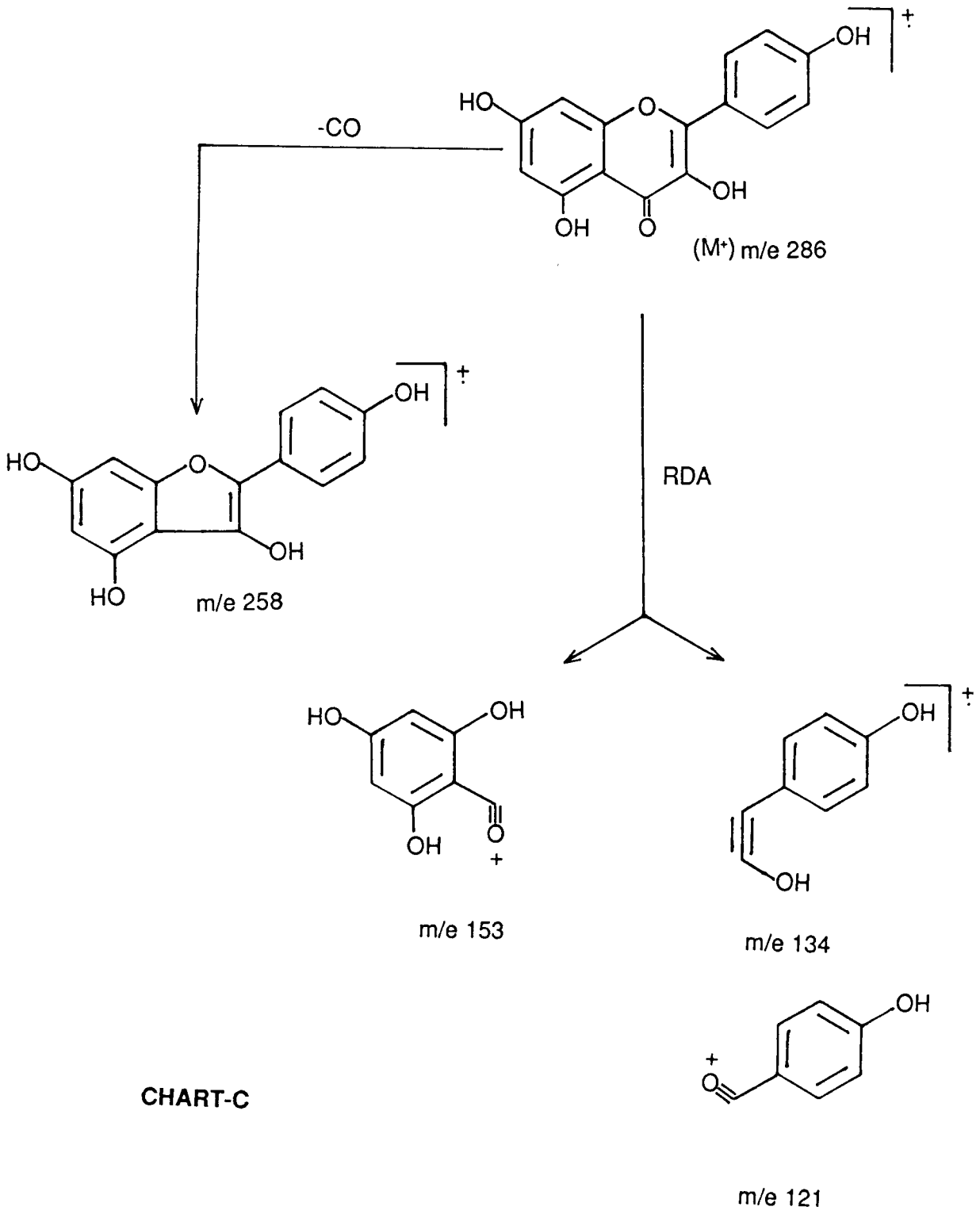


CHART-C

did not give any appreciable quantities of the products which could be studied and hence further studies were not carried out. The ethyl acetate extract on usual work up gave a yellow crystalline compound, m.p. 248-50° which was glycosidic in nature and was T.L.C. and paper chromatography pure. It gave positive tests for flavonoids. It was coded as **KIL-3**. On acetylation with acetic anhydride and pyridine it gave an acetyl derivative, m.p. 110° which was coded as **(KIL-3A).**

On hydrolysing the compound KIL-3 by killiani's mixture and usual processing a yellow crystalline compound, m.p. 245° was obtained which gave a positive test for flavonoid. Its U.V. spectrum and spectral shifts were close to those of KIL-2 and m.m.p. did not show any depression. These data suggested that the aglycone is 3,5,7,4'-tetra hydroxy flavone.

The U.V. spectrum of the glycoside showed two bands at 266.8 and 351 nm. Addition of $AlCl_3$ shifted the longwave length band to 396 nm.which was not affected by HCl, indicating the presence of a hydroxyl group at 5 position. Sodium acetate produced a bathochromic shift of 8 nm. in Band II suggesting the presence of 7-OH group. Taken together with the location of band I at 351 nm.,it could be concluded that the compound is a 5,7 dihydroxy flavonol with 3-hydroxyl being protected in the form of glycoside.

The NMR spectrum of the above acetate (KIL-3A) showed four alcoholic acetoxyls between δ 1.9 and δ 2.1 and three phenolic acetoxyls as two singlets at δ 2.35 (6H) and δ 2.45 (3H) and a signal for $-CH_2$ moiety of sugar at δ 3.85. In the aromatic region there was one proton meta coupled doublet at δ 6.8 (J = 2 Hz) for H-6 and a two proton doublet centred at δ 8.0 (J = 9 Hz) for two protons characteristic of H-2',6' . The other doublet of the A_2B_2 system was located at δ 7.3 overlapping with the signal from H-8. Besides these there were signals from carbinyl protons of the sugar moiety from δ 5.0 to δ 6.0. These data suggested that the compound may be the acetate of kaempferol-3-glycoside.

A further support to the above structure was obtained by the mass spectral studies of the glycoside acetate (KIL-3A) which did not show the molecular ion peak but showed a peak at m/e 412 corresponding to molecular ion of 4',5,7 triacetoxy flavonol formed from the parent ion by the loss of sugar moiety. The other important peaks observed were at m/e 370, 328, 286, 258, 153, 134 and 121. The fragmentation pattern has been shown in the **Chart 'D'.**

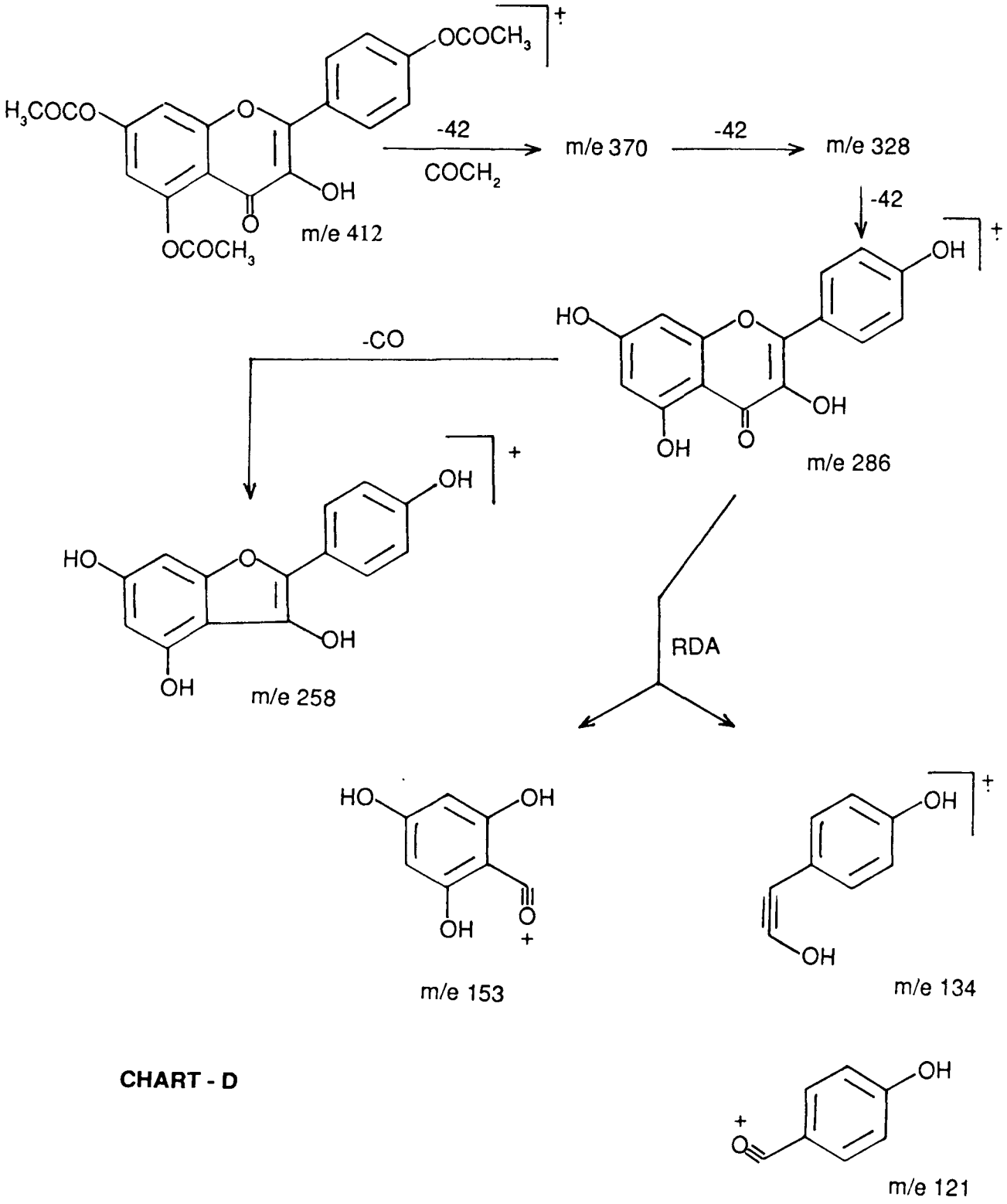

H3COCO
OCOCH3
OCOCH3
OH
O
m/e 412
-42
COCH2
m/e 370
-42
m/e 328
-42
HO
OH
OH
O
m/e 286
-CO
HO
OH
OH
OH
m/e 258
RDA
HO
OH
OH
O
m/e 153
OH
OH
m/e 134
OH
O
m/e 121

CHART - D

The aqueous layer of the hydrolysate left after removal of the aglycone by ether extraction was evaporated to dryness and tested for the identification of the sugar moiety by paper chromatography. Authentic samples of different sugars were applied on the paper chromatogram alongwith the test material. The spot of the test material corresponded well with the spot of galactose and hence the sugar was identified as galactose and the parent glycoside was identified as **kaempferol-3-galactoside (Trifolin)**. A literature survey showed that physical and spectral properties of this compound are in agreement with those reported for trifolin[15].

ISOLATION AND CHARACTERISATION OF KIL-4 FROM BUTANOL EXTRACTS OF F-3

The butanol extract after concentration was left in a refrigerator for a week's time. A brown solid deposited in the bottom of the flask which after purification gave a colourless compound **(KIL-4)**, m.p.163-64°. It was sweet in taste,did not reduce Fehling's solution and gave a positive test for the presence of polyhdric alcohols[16]. On acetylation it gave an acetate**(KIL-4A)** m.p.110°. On the basis of the identical physical properties of the parent compound and its acetate with those of mannitol and its hexaacetate, (m.p., m.m.p. and paper chromatography), it was identified as **mannitol**. The identity as mannitol further confirmed by superimposable I.R. Spectra.

EXPERIMENTAL

STUDY OF THE LEAVES OF *ICHNOCARPUS FRUTESCENS* R.Br. SYN.,*APOCYNUM FRUTESCENS* (N.O. APOCYNACEAE)

EXTRACTION AND SEPARATION INTO PETROL SOLUBLE (F-1), WATER INSOLUBLE (F-2) AND WATER SOLUBLE (F-3) FRACTIONS.

The leaves of *I. frutescens* were collected from a neighbouring village, Sanai, of Tehsil Bilari in District Moradabad (U.P.) and dried under shade. The dried leaves (1.0 kg) were exhaustively extracted with hot ethanol (95%) five times. All the ethanolic extracts were combined together. Recovery of the solvent left a greenish viscous mass (100 gms). The alcoholic concentrate was extracted with petroleum ether (60-80°) several times to remove the petrol soluble part (25 gm). It was coded as **F-1**. The residue left after petrol extraction was taken up in water and filtered to give the water insoluble fraction, **F-2** (30 gm) and water soluble fraction, **F-3** (45 gm). The water insoluble fraction, (**F-2**), was extracted successively with $CHCl_3$ and solvent ether which on evaporation to dryness gave (3.0 gm) and (5.0 gm) of the concentrates respectively. The water soluble fraction (**F-3**) was successively extracted in a liquid-liquid extractor with petrol (60-80°), solvent ether, ethyl acetate and butanol.

STUDY OF THE PETROL SOLUBLE FRACTION (F-1), ISOLATION OF KIL-1

The petroleum ether fraction (**F-1**, 25 gm) after redissolving in petroleum ether and concentrating to a small volume was left in a refrigerator overnight. A green solid (1.2 gm) separated out, was filtered and washed with petroleum ether (60-80°). It gave a positive Liebermann-Burchard test (red colour) for triterpenes. On T.L.C. examination in toluene, ethyl acetate (7:3), it showed two spots (one major and one minor). All attempts to purify this fraction through crystallisation with different organic solvents did not succeed and hence a recourse was taken to other methods for the purpose of purification.

ACETYLATION

The above compound (1.2 gm) was dissolved in dry pyridine (4 ml) and acetic anhydride (3 ml) was added to it. The contents were heated on a boiling water bath for two hours, left overnight at room temperature and then processed in the usual way to give the crude product which was

dissolved in methanol and treated with activated animal charcoal. A clear light brown filtrate so obtained was concentrated to about 25 ml and left at room temperature but it did not crystallise out.

PURIFICATION OF THE ACETATE TO GIVE KIL-1

The above filtrate was evaporated to dryness and after dissolving in minimum quantity of ethyl alcohol (2 ml) extracted three times with hot petroleum ether (60-80°) using 100 ml of petrol each time. All the petrol extracts after combining together were evaporated to dryness to give a colourless semi solid mass which crystallised from methanol to give a T.L.C. pure (solvent system; toluene: ethyl acetate; 8:2) colourless crystalline compound **KIL-1**, (m.p. 292°, 300 mg).

SPECTRAL DATA (KIL-1)

^{1}HNMR (δ, $CDCl_3$)

0.7-1.0 ($7xCH_3$), 2.0 (s, 3H, $OCOCH_3$), 4.46(m, 1H, H-3), 5.26 (m, 1 H, H-12).

Mass (m/e)

498 (M^+), 438, 249, 248, 203, 189.

METHYLATION OF THE ACETATE KIL-1 TO GIVE KIL-1 AM

The acetate (100 mg) was methylated with an excess of ethereal solution of diazomethane and left at room temperature. It was filtered next morning and the ethereal solution evaporated to dryness. The residue on crystallisation with methanol gave a colourless crystalline compound **KIL-1AM** (80 mg, m.p. 210°).

^{1}HNMR (δ, $CDCl_3$) (KIL-1 AM)

0.72-1.02 ($7xCH_3$), 2.0 (s, 3H, $OCOCH_3$), 3.6(s, 3H, $COOCH_3$), 4.40 (m, 1 H, H-3), 5.20 (m, 1H, H-12).

Mass (m/e)

512(M^+), 452, 262, 249, 203, 189.

DEACETYLATION OF THE ACETATE KIL-1

The acetate **KIL-1** (50 mg) was refluxed for four hours with methanolic sodium hydroxide (25 ml, 4%) on a water bath. After evaporating off half of the solvent, the contents were diluted with water (50 ml) which did not give any insoluble product. It was therefore acidified with HCl to give colourless precipitate which was filtered, washed with water till free from acid and dried. The filtrate was extracted with solvent ether (2x50 ml) in a separating funnel. The two ethereal extracts were combined together, washed with water to remove acid and dried over anhydrous sodium sulphate. After filtering off the inorganic salt, ether was distilled off. A residue in very small quantity so obtained after T.L.C. check up was combined together with the precipitate obtained above. On crystallisation from methanol it gave a T.L.C. pure colourless crystalline compound (20 mg, m.p. 286-87°). By running its T.L.C. in petroleum ether: ethyl formate: formic acid (93:7:0.7) alongside with an authentic sample of ursolic acid and oleanolic acid, it was found to be identical with ursolic acid (Rf 0.19). m.m.p. 286°.

STUDY OF THE $CHCl_3$ SOLUBLE FRACTION FROM F-2 (FURTHER ISOLATION OF KIL-1)

Recovery of chloroform left a greenish semi solid mass (3 gm) which gave a positive L.B. test (red colour). After dissolving in solvent ether (25 ml), petroleum ether (25 ml) was added to it and the contents left at room temperature for 48 hours when a solid separated out. It was filtered, washed with petrol and acetylated with pyridine and acetic anhydride in the cold and worked up as usual. The crude acetate was purified in the same way as given above. It gave some more quantity of **KIL-1** (ursolic acid acetate, 70 mg, m.p. 290-91°).

STUDY OF THE SOLVENT ETHER SOLUBLE FRACTION FROM F-2 (ISOLATION OF KIL-2)

Recovery of the solvent ether left a solid brown mass (5 gm) which gave a pink colour with Mg-HCl, a green colour with alcoholic $FeCl_3$ and an intense yellow colour on exposure to ammonia vapours, indicating the presence of flavonoids. It was subjected to column chromatography on silica gel (60-120 mesh, 100 gm, diameter of the column 2.5 cm). The column was successively eluted with

petrol (500 ml), benzene (500 ml), benzene-ethyl acetate mixtures in different proportions (9:1, 3:1, 1:1, 500 ml each) and finally with pure ethyl acetate (500 ml). The first two fractions of 100 ml benzene-ethyl acetate mixture (9:1) showed a single brown spot on T.L.C. examination under U.V. light. These fractions were combined together and evaporated to dryness. The residue was dissolved in acetone (3 ml) and benzene (10 ml) was added to it. It was left at room temperature. After two days a yellow solid separated out which was filtered and crystallised from aqueous methanol to give a yellow crystalline compound **KIL-2** (m.p. 246-47°, 100 mg) which gave a pink colour with Mg-HCl, a greenish blue colour with alcoholic $FeCl_3$ and an intense yellow colour on exposure to ammonia vapours.

SPECTRAL DATA (KIL-2)

U.V. (Methanol)

λ_{max}^{MeOH} 268, 294 (inf), 322 (inf), 368 nm.

$\lambda_{max}^{AlCl_3}$ 260(inf), 268, 303 (inf), 350, 421 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 269, 303 (inf), 348, 421 nm.

λ_{max}^{NaOAc} 275, 303, 387 nm.

$\lambda_{max}^{NaOAc+Boric\ Acid}$ 267, 297 (inf), 320 (inf), 371 nm.

Mass (m/e)

286 (M^+), 258, 153, 134, 121.

ACETYLATION OF KIL-2

The compound **KIL-2** (75 mg) was dissolved in pyridine (1.0 ml) and acetic anhydride (1.0 ml) was added to it. The contents were heated on a boiling water bath for two hours and left at room temperature overnight. The reaction mixture was then worked up as usual to give a crude product which was crystallised from methanol to give a colourless T.L.C. pure compound **KIL-2A** (50 mg, m.p. 118°). It was identified as kaempferol acetate on the basis of NMR spectral data and T.L.C. comparison with an authentic sample.

SPECTRAL DATA (COMPOUND KIL-2A) ^{1}HNMR (δ, $CDCl_3$)

2.3 (s, 9H, 3x$OCOCH_3$), 2.4 (s, 3H, $OCOCH_3$), 6.8 (d, J=2 Hz, H- 6), 7.7 (d, J=9Hz, 2H, H-2', H-6'), 7.3 (m, 3H, H-8, 3',5').

STUDY OF THE WATER SOLUBLE FRACTION (F-3)

The water soluble fraction, **F-3** was redissolved in water and subjected to liquid-liquid extraction with petrol (60-80°), solvent ether, ethyl acetate and butanol successively. The petrol (60-80°) and solvent ether fractions did not yield any appreciable quantities of the products and hence no further studies were carried out on these fractions.

STUDY OF THE ETHYL ACETATE FRACTION (ISOLATION OF KIL-3)

The ethyl acetate layer was dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The residue was extracted with hot dry acetone and filtered. The acetone solution was concentrated to a small volume and left at room temperature when a yellow crystalline compound **KIL-3** (m.p. 248-50°, 200 mg) was obtained which was T.L.C. and paper chromatography pure in the solvent systems chloroform, methanol, water; 70:27:3.6 and butanol, acetic acid, water; 4:1:5 respectively. It gave a pink colour with Mg/HCl, a green colour with $FeCl_3$, a brown flourescence under U.V. light, and also gave a positive Molisch test.

SPECTRAL DATA (COMPOUND KIL-3)

U.V. (MeOH)

λ_{max}^{MeOH} 266.8, 351 nm.

$\lambda_{max}^{AlCl_3}$ 274, 303.2, 350, 396 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 273.5, 302.8, 345.6, 394.4 nm.

λ_{max}^{NaOAc} 274.5, 301.7, 338.5 (inf), 371.8 nm.

$\lambda_{max}^{NaOAc+Boric\ Acid}$ 266.6, 302.3 (inf), 351.2 nm.

ACETYLATION OF KIL-3

The compound (100 mg) was dissolved in pyridine (2 ml) and acetic anhydride (1.0 ml) was added to it. The contents after leaving at room temperature for 24 hours were processed as usual to give a light brown product which could not be crystallised.

PURIFICATION OF THE ACETATE

The acetate after dissolving in minimum quantity of ethyl alcohol (0.5 ml) was extracted with hot petroleum ether (60-80°) 3x25 ml. All the petrol extracts were combined together and concentrated to a small volume to give a colourless compound (**KIL-3A**), m.p.110°, 25 mg.

SPECTRAL DATA (COMPOUND KIL-3A)

^{1}HNMR (δ, $CDCl_3$)

1.9-2.1 (12 H, 4x$OCOCH_3$), 2.35 (s, 6H, 2x$OCOCH_3$), 2.45 (s, 3H, $OCOCH_3$), 3.85 (-CH_2), 5.0-6.0 (m, carbinyl protons), 6.8 (d, J=2Hz, H-6), 7.3 (m, 3H, H-8, 3',5'), 8.0 (d, J=9Hz, H-2',H-6').

MASS (m/e) (COMPOUND KIL-3A)

412, 370, 328, 286, 258, 153, 134, 121.

HYDROLYSIS OF KIL-3

The compound **KIL-3** (20 mg) was hydrolysed in a test tube by Killiani's mixture (1.0 ml) by heating in a boiling water bath for two hours. The reaction mixture was processed as given on page 37-38. The ethereal layer on evaporation gave a yellowish brown solid mass which was crystallised from methanol (5 mg, m.p. 245°). It gave a red colour with Mg/HCl, an intense yellow colour on exposure to NH_3 vapours and a brown flourescence under U.V. light. Its identity as Kaempferol was established by mixed melting with an authentic sample of Kaempferol (m.m.p. 245°), and co-T.L.C. examination (toluene, ethyl formate, formic acid; 5:4:1, toluene, chloroform, acetone; 8:5:7).

SPECTRAL DATA

U.V. (MeOH)

λ_{max}^{MeOH} 267, 294 (inf), 320 (inf), 368 nm.

$\lambda_{max}^{AlCl_3}$ 260 (inf), 268, 303 (inf), 350, 423 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 269, 303 (inf), 348, 423 nm.

λ^{NaOAc}_{max} 275, 303, 387 nm.

$\lambda^{NaOAc+Boric\ Acid}_{max}$ 267,297 (inf), 320 (inf), 371 nm.

IDENTIFICATION OF SUGAR

The aqueous layer left after extraction with ether (Killiani hydrolysis of **KIL-3**) was evaporated to dryness in a china dish on a water bath. The residue was dissolved in a few drops of water and co-chromatographed with different sugars (as described on page 38). It was identified as galactose. On the basis of above data the compound **KIL-3** was identified as kaempferol-3-galactoside.

STUDY OF BUTANOL FRACTION (ISOLATION OF KIL-4)

The butanol extract (500 ml) after concentrating under reduced pressure to one third of its original volume was left in the refrigerator for a week. A light brown solid (1.0 gm) deposited in the bottom of the flask which was filtered and washed with butanol. After dissolving in hot water (6.0 ml), ethanol (100 ml) was added to it and the contents were charcoaled under boiling conditions and filtered. The filtrate was concentrated and left at room temperature to give a colourless crystalline compound **KIL-4** (m.p. 163-64°, 300 mg) which was sweet in taste and did not reduce Fehling's solution. It gave a positive test for polyhydric alcohols (added a small amount of the compound to an aqueous solution of 1% borax containing two drops of phenolphthalein, the pink colour disappeared which reappeared on warming and vanished again on cooling). On paper chromatographic examination by descending technique in, ethyl acetate, pyridine, water; 7:5:2 alongwith an authentic sample of mannitol, its spot corresponded well with mannitol. The chromatogram was run at room temperature for 48 hours and developed in iodine vapours. On mixed melting point with an authentic sample, no depression in melting point was observed (m.m.p. 163-64°). Its I.R. spectrum was superimposable with the I.R. spectrum of mannitol.

ACETYLATION OF KIL-4

The compound **KIL-4** (200 mg) was refluxed with acetic anhydride (5 ml) and fused sodium acetate (300 mg) for two hours. After cooling to room temperature the reaction mixture was

poured dropwise into ice cold water and worked up in the usual way. On crystallisation with dichloromethane-petroleum ether (60-80°) mixture, it gave a colourless compound (**KIL-4A**) (100 mg, m.p. 110°). It was identified as Mannitol acetate by superimposable spectra.

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CHAPTER - III

DISCUSSION

STUDY OF THE AERIAL PARTS OF *SISYMBRIUM IRIO* LINN. (N.O. CRUCIFERAE)

VERNACULAR NAMES

Hindi - Khub Kalan, **English** - London rocket, **Punjab** - Maktrusa, Jangli Sarsoon, **Bombay** - Khakshi, **Raj.** - Parjan, **Mar.**- Ran - tikhi, **Pers.** - Khakshir, **Arab** - Khubah.

DISTRIBUTION

It is found in North - West India and North - West temperate Himalayas.[1]

BOTANICAL DESCRIPTION

It is an annual herb, highly variable, 20-60 cm tall, stems are 2-10 mm thick. Leaves are pinnatifid or partite and lobes are distant. Flowers are yellow, pods narrow, erect and subtorulose. It is found abudantly in the winter season on moist soil in waste places, in fallow and cultivated fields. Flowering and fruiting take place during Feb. - April.[2]

MEDICINAL PROPERTIES AND USES

Leaves are edible and are eaten either raw or cooked. They are rich in vitamin C (176 mg/ 100 gm), β -carotene (10,000 IU/100 gm) and minerals. Seeds are expectorant, febrifuge and rubefacient. The seeds are also used in asthma and employed in the preparation of stimulating poultices and yield a semi-drying oil which is used for soap - making and lubrication.[3]

LITERATURE SURVEY

Earlier investigations[4] in our labs, gave interesting biological results. The extracts of seeds prepared by successive extraction with light petroleum ether, ethanol and distilled water were tested for antipyretic, analgesic and antimicrobial activity. The ethanolic extract showed significant antipyretic effect in yeast induced pyrexia in albino rats and significant analgesic activity was also observed in acetic acid induced writhing in mice. The ethanolic and aqueous extracts exhibited antibacterial activity against both gram positive and gram negative organisms; however, these

effects were particularly marked with the ethanolic extract. The petroleum ether and aqueous extracts did not show antipyretic and analgesic activity. All these extracts were found to be devoid of antifungal activity against *Candida albicoms, Microsporum canis, M. gypseum, Trichophyton rubrum* and *Philophora jeanselmei*, and they did not have any toxicity upto a dose level of 2 gm/ kg. P.O. in albino mice.

The seeds have been reported[5] to contain 18-20 percent of a brownish yellow oil having following characteristics: specific gravity at 40° - 0.9083, viscosity at 40° - 40.3 centistokes, refractive index at 30° - 1.4672, acid value - 3.1, saponification value - 176.5, iodine value (wijs) - 109.4, thiocyanogen value - 78.7, Hehner value -94.8, acetyl value - 11.9, unsaponifiable matter 1.6% , Reichert - Meissl value - 0.05, hexa bromide value - 3.1, soluble fatty acids - 0.4%, saturated fatty acids - 11.1%, fatty acids are: erucic acid - 17.6%, oleic acid - 27.5%, linoleic acid 35.25%, linolenic acid - 8.3%, palmitic acid - 8.15%, stearic acid - 3.1% and arachidic acid - 0.1%. The unsaponifiable fraction of the oil contained sitosterol.[5]

A flavonoid was isolated from the defatted seeds by alcoholic extraction and identified as isorhamnetin by direct comparison with an authentic sample (co-chromatography, m.m.p. U.V. spectroscopy.[6]

The leaves have been investigated for carotenes.[7] 24-methylene-cholesterol was identified in its pollens by mass spectroscopy.[8]

PRESENT WORK

The aerial parts of the plant were collected from the Hamdard Nagar Campus, New Delhi, dried in the shade, and chopped into small pieces. The dried material was exhaustively extracted with ethanol and the ethanolic concentrate was extracted with petroleum ether to remove the petrol soluble part. The petrol insoluble residue was taken up in water and filtered to separate the water soluble and water insoluble fractions.

ISOLATION OF KSI-1 AND KSI-2 FROM PETROL SOLUBLE PART

The residue left after recovery of petrol gave a positive Liebermann - Burchard test showing the presence of steroids. On T.L.C. examination, it was found to be a mixture and was purified by

column chromatography. The column on elution with petroleum ether-benzene (3:1) mixture gave a solid which on repeated crystallisation with methanol gave a colourless T.L.C. pure compound **KSI-1**, m.p. 137°. It was identified as **β-sitosterol** by T.L.C. comparison m.m.p. and superimposable I.R. spectra. On acetylation it gave an acetate (**KSI-1A**), m.p. 126° which was also identical with β-sitosterol acetate (m.m.p. and co-T.L.C. examination).

Further elution of the column with acetone gave a cream coloured solid which on repeated crystallisation with methanol gave a colourless compound, m.p. 270-80°. On T.L.C. examination it was found to be a mixture of two components (one major and one minor). On hot acetylation in the usual way it gave an acetate which on crystallisation with methanol gave a colourless T.L.C. pure compound **KSI-2** m.p. 150°. It gave a positive L.B. test.

The NMR spectrum of the compound showed the presence of 6 methyl functions in the range of δ 0.678 to δ 0.987. The presence of four acetyl groups at δ 2.019 (s, 6H), δ 2.049 (s, 3H) and δ 2.078 (s, 3H) suggested it to be a monoglyscosidic acetate. It showed a signal around δ 5.32 characteristic of an olefinic proton of Δ^5 steroids. It showed a group of multiplets from δ 4.16 to δ 5.13 for protons α to acetoxyls reminiscent of monoglycoside acetate. From these data it could be concluded that the present compound is a steroidal monoglycoside. The above acetate on deacetylation with methanolic sodium hydroxide gave a T.L.C. pure colourless crystalline compound **(KSI-2DA)** m.p. 280°. It was subjected to Killiani's hydrolysis and then after dilution with water extracted with ether in order to isolate the aglycone. Recovery of ether left a small residue which was found to be identical with β-sitosterol on the basis of co-T.L.C. examination.

The aqueous layer after evaporating to dryness was dissolved in a few drops of water. It gave positive test for reducing sugars. By paper chromatographic examination, the sugar was identified as glucose.

On the basis of the above data the compound **KSI-2** was identified as tetraacetate **β-D-glucoside of β-sitosterol**. A comparison of the melting points of deacetylated compound (KSI-2DA) and β-D-glucoside of sitosterol, m.p. 280°, further established its identity as β-sitosterol-3β-D-glucoside.

HYDROLYSIS OF WATER SOLUBLE FRACTION

The water soluble fraction was hydrolysed by dilute sulphuric acid. The aglycone so obtained was filtered, washed with water and dried. It was exhaustively extracted with solvent ether. Recovery of solvent left a dark brown residue which was chromatographed on a column of silica gel and eluted with petrol, benzene, benzene-ethyl acetate mixtures in different proportions and finally with ethyl acetate. Benzene-ethyl acetate (3:1) eluate on T.L.C. examination in (toluene, ethyl formate, formic acid; 5:4:1) showed two brown spots with Rf values 0.61 and 0.63. This mixture was separated by preparative T.L.C. on silica gel G plates. The compounds from upper and lower bands of T.L.C. plates were marked as **KSI-3** and **KSI-4** respectively and eluted separately in two different flasks.

CHARACTERISATION OF KSI-3

The product **KSI-3** on crystallisation from methanol gave a yellow crystalline compound, m.p. 303-304° which gave positive tests for flavonoids. On acetylation it gave an acetyl derivative **(KSI-3A)**, m.p. 202°.

The U.V. spectrum of the compound showed two maxima at 255.4 nm.(Band II) and 372.0 nm (Band I) with two inflexions at 267.38 nm and 326.5 nm. Addition of sodium acetate shifted Band II to 274.0, which suggested a free hydroxyl group at 7 position. In sodium acetate/boric acid the spectrum shifted to the parent compound spectrum in having the maxima at 255.5 nm. and 372.0 nm.,thus suggesting the absence of any catecholic group.

Addition of $AlCl_3$ shifted band I to 429 nm. This suggested the presence of a free hydroxyl group at the 5 position. The location of band I at 372 nm.suggested that there is an - OH group at 3 position also. Addition of HCl to $AlCl_3$ containing solution did not produce any drastic change which also supported our findings.

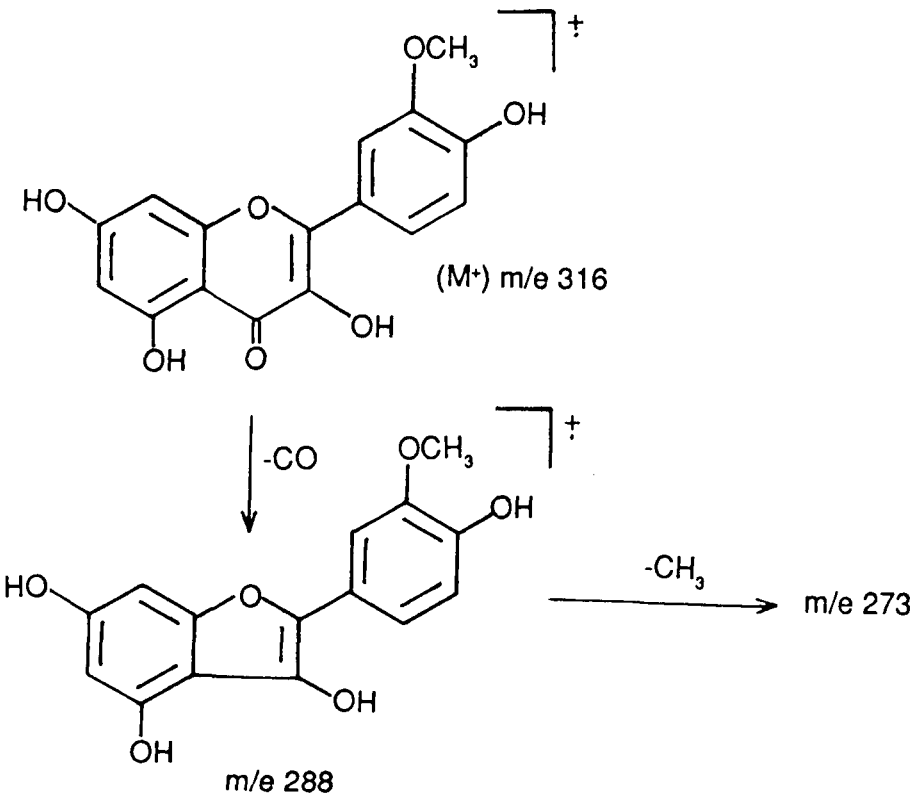
The above data suggested hydroxyl groups at 3,5,7 and 4' positions. The position of band-I at 372.0 nm.is also indicative of the presence of 3 and 4' hydroxyl groups.

The NMR spectrum of the compound showed four acetoxyls as two singlets at δ 2.33 (9H) and δ 2.53 (3H) and a methoxyl at δ 3.9 indicating that the compound has one methoxyl and four

acetoxyl functions. In the aromatic region there was a meta coupled doublet for one proton at δ 6.9 and a multiplet from δ 7.2 to δ 7.6 for four protons. The former can be assigned to H-6 and its appearance suggested that the compound is a 5,7-dioxygenated flavonoid. These data suggest that the compound could be rhamnetin or isorhamnetin. A careful study based on physical properties showed that the compound is isorhamnetin.

A further support to this compound being isorhamnetin, was obtained by mass spectral studies. The mass spectrum (**Chart**) showed the molecular ion peak at m/e 316 consistent with the molecular formula $C_{16}H_{12}O_7$. There was a peak at m/e 288 for a loss of CO from the molecular ion as is normally observed in the mass spectra of flavonoids. There was also a peak of low abundance at 273. A T.L.C. comparison of the compound with an authentic sample established its identity as **isorhamnetin**.

CHART



CHARACTERISATION OF KSI-4

KSI-4 on crystallisation from methanol gave a yellow compound, m.p. 310-11° which gave positive tests for flavonoids. The mass spectrum of the compound showed the molecular ion peak at m/e 302 corresponding to $C_{15}H_{10}O_7$. There was a loss of CO from the molecular ion peak to give a peak at m/e 274 which is typical of flavonoids.

The U.V. spectrum of the compound showed two maxima at 256 and 370 nm with an inflexion at 301 nm. Addition of $AlCl_3$ shifted band I to 456 nm. This suggested the presence of a free hydroxyl group at the 5 position. The location of band I at 370 nm.suggested that there is a hydroxyl group at 3 position also. Addition of HCl to $AlCl_3$ changed the long wave length maxima to 426 nm. This is known in the case of catecholic systems in which the corresponding aluminium complex breaks down with HCl.

By adding sodium acetate to the methanolic solution, the band II shifted to 273 nm, thus suggesting the presence of a hydroxyl group at 7 position. In $NaOAc/H_3BO_3$ the band I shifted to 388 nm.,showing a bathochromic shift of 18 nm.,suggesting the presence of a catecholic unit in the side phenyl. Thus the compound appears to have hydroxyls at 3, 5, 7, 3', 4' positions.

The compound (KSI-4) on acetylation gave an acetate (**KSI-4A**) m.p. 195-96°. The NMR spectrum of acetate showed 5 acetoxyls, four of them as a singlet δ 2.4 and the fifth as a separate singlet at δ 2.5. In the aromatic region there was an unresolved one proton signal at δ 7.0 characteristic of H-6 and another unresolved one proton signal at δ 7.5 for H-8. There was a two protons multiplet centred at δ 7.85 for H-2' and 6' and a doublet (J = 8 Hz) for one proton at δ 7.3 possibly from H-5. These data agree for quercetin pentaacetate and hence the parent compound is **quercetin.**

EXPERIMENTAL

STUDY OF THE AERIAL PARTS OF *SISYMBRIUM IRIO* LINN. (N.O. CRUCIFERAE)

EXTRACTION

The fresh material was collected from the Hamdard Nagar Campus, New Delhi and chopped into small pieces after drying under shade. The dried material (3 kg) was exhaustively extracted by refluxing with ethanol (95%) five times. All the ethanolic extracts were combined together. Recovery of solvent left a greenish viscous mass which was extracted in hot with petroleum ether (60-80°) several times in order to isolate petrol soluble compounds. The residue left after petrol extraction was taken up in water and filtered to give water soluble and water insoluble fractions.

STUDY OF THE PETROL SOLUBLE FRACTION

It gave a green colour by Liebermann Burchard test. A T.L.C. examination of this fraction in n-hexane, toluene, ethyl acetate (10:5:3); toluene, ethyl acetate (7:3); and benzene, methanol (8:2) showed it to be a mixture of more than two components.

PURIFICATION BY COLUMN CHROMATOGRAPHY

The petrol soluble fraction (15 gm) was chromatographed on a column of silica gel (300 gm, 60-120 mesh, diameter of the column 2.5 cm) and eluted with petroleum ether (60-80°, 1 lit), petrol-benzene mixture (3:1 and 1:1, 500 ml each), benzene (500 ml) and finally with acetone (500 ml). Petrol, petrol - benzene (1:1) and benzene eluates could not be purified and were not investigated further.

STUDY OF PETROL-BENZENE (3:1) ELUATE AND ISOLATION OF THE COMPOUND KSI-1

It was evaporated to dryness and the residue on crystallisation from methanol gave a light yellow compound which was repeatedly crystallised from methanol to give a colourless compound **KSI-1**, m.p. 137° (100 mg). On T.L.C. examination in n-hexane, toluene, ethyl acetate (10:5:3) and toluene, ethyl acetate (9:1) it was found to be a single entity. It was identified as β-sitosterol by m.m.p. (137°), co-T.L.C. examination and superimposable I.R. spectra.

ACETYLATION OF KSI-1

The compound (70 mg) was dissolved in pyridine (1.0 ml) and acetic anhydride (1.0 ml) was added to it. The contents were heated on a boiling water bath for two hours and processed in the usual way. The crude acetyl derivative on crystallisation from methanol gave a colourless crystalline compound (**KSI-1A**) , m.p. 126° (30 mg). It was identical with β-sitosteryl acetate (co-T.L.C. examination, superimposable I.R. spectra and m.m.p. 126-27°).

STUDY OF ACETONE ELUATE

The acetone fraction eluted from the column was concentrated to a small volume and left at room temperature to give a cream coloured compound which was filtered and recrystallised from methanol. A colourless compound, m.p. 270-280° (150 mg) was obtained. It gave a green colour by L.B. test. On T.L.C. examination in benzene - methanol (9:1) it appeared to be a mixture of at least two components (one major and one minor).

ACETYLATION OF THE ABOVE COMPOUND TO GIVE (KSI-2)

The compound (100 mg) was dissolved in pyridine (2.0 ml) and acetic anhydride (1.0 ml) was added to it. The contents were heated on a boiling water bath for two hours and left at room temperature for 24 hours. The reaction mixture was then processed in the usual way to give the crude acetyl derivative which was crystallised from methanol to give a colourless compound **KSI-2** (60 mg, m.p. 150°). It gave a green colour by L.B. test. On T.L.C. examination in toluene, ethyl formate, formic acid (5:4:1), it was found to be a single entity.

SPECTRAL DATA

^{1}HNMR (δ, $CDCl_3$)

0.678-0.987 (6xCH_3), 2.019 (s, 6H, 2x$OCOCH_3$), 2.049 (s, 3H, $OCOCH_3$), 2.078 (s, 3H, $OCOCH_3$), 4.16 - 5.13 (m, 7H protons α to acetoxyls), 5.32 (m, 1H, H-5).

DEACETYLATION OF THE ABOVE ACETATE

The acetate (**KSI-2**) (40 mg) was refluxed with methanolic sodium hydroxide (10 ml, 5%) on a boiling water bath for three hours and then processed as usual to give a colourless crystalline compound (**KSI-2DA**) m.p. 280°, 20 mg. On T.L.C. examination in (benzene - methanol, (9:1), it showed a single spot.

KILLIANI'S HYDROLYSIS OF THE ABOVE DEACETYLATED PRODUCT

The compound (**KSI-2DA**) (10 mg) was taken in a test tube and Killiani's mixture (1.0 ml) was added to it. On usual work up as described on page 37-38, the ethereal layer gave a small residue which by running its T.L.C. alongwith an authentic sample of β-sitosterol in the solvent systems (toluene-ethyl acetate, 9:1) and n-hexane, toluene, ethyl acetate; (10:5:3) was identified as β-sitosterol.

IDENTIFICATION OF THE SUGAR

The aqueous layer left after extraction with ether (Killiani's Hydrolysis) was evaporated to dryness in a china dish on a water bath to give a residue of sugar. On co-chromatography with different sugars (as described on page 38), it was identified as glucose.

STUDY OF THE WATER SOLUBLE FRACTION

The water soluble fraction was subjected to hydrolysis with dil. H_2SO_4 (4% v/v) by heating on a boiling water bath for four hours and finally the hydrolysis was completed by refluxing on a wire gauze for another one hour. A dark brown precipitate (6.0 gm) separated out which was filtered, washed with water several times till free from acid and dried. It gave a pink colour with Mg/ HCl and a yellow colour on exposure to ammonia vapours which indicated the presence of flavonoids.

PURIFICATION OF THE ABOVE AGLYCONE

The above crude aglycone was extracted with solvent ether several times. Recovery of solvent left a dark brown residue (3.0 gm). It was subjected to column chromatography on a glass

column (diameter 2.5 cm) on silica gel (60-120 mesh, 60 gm). The column was eluted with petrol (60-80°) (500 ml), benzene (500 ml), benzene - ethyl acetate mixtures (3:1, 1:1, 1.5 lit each) and finally with ethyl acetate (1.0 lit). Benzene-ethyl acetate (3:1) eluate (1.5 lit) was evaporated to dryness to give a pale yellow residue (1.0 gm). On running its TLC in toluene: ethyl formate: formic acid (5:4:1) solvent system and exposure to U.V. light it showed two brown spots with close Rf values (Rf 0.61 and 0.63).

SEPARATION OF COMPOUNDS KSI-3 AND KSI-4 BY PREPARATIVE T.L.C.

The above mixture was separated by preparative T.L.C. on silica gel - G plates using toluene: ethyl formate: formic acid (5:4:1) as solvent system. About 200 plates (size 20x20 cm) were run. The two bands (upper and lower) were carefully separated from the plates, and transferred into two separate small columns. On elution with methanol and evaporation of the solvent two fractions from the upper and lower bands were obtained.

KSI-3 (COMPOUND ISOLATED FROM UPPER BAND)

On crystallisation from methanol, it gave a yellow crystalline compound, m.p. 303-304° (50 mg) which gave pink colour with Mg- HCl, an intense yellow colour on exposure to ammonia vapours, and a greenish colour with alcoholic $FeCl_3$ solution.

SPECTRAL DATA

UV (Methanol)

λ_{max}^{MeOH} 255.4, 267.38 (inf), 326.5 (inf) 372.0 nm.

$\lambda_{max}^{AlCl_3}$ 242, 263, 304 (inf), 361.4 (inf), 429 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 242, 264, 302 (inf), 359, 428 nm.

λ_{max}^{NaOAc} 274, 320, 393 nm.

$\lambda_{max}^{NaOAc+H_3BO_3}$ 255.5, 270 (inf), 326 (inf), 372 nm.

Mass (m/e)

316 (M^+), 288, 273

ACETYLATION OF KSI-3

The compound **KSI-3** (35 mg, m.p. 303-304°) was dissolved in pyridine (0.5 ml) and acetic anhydride (0.5 ml) was added to it. The contents were left at room temperature for 24 hours and then worked up as usual to give the crude acetate which on crystallisation with methanol containing a few drops of chloroform gave colourless crystals (**KSI-3A**) (m.p. 202°, 23 mg).

SPECTRAL DATA

^{1}HNMR (δ, $CDCl_3$)

2.33 (s, 9H, 3x$OCOCH_3$), 2.53 (s, 3H, $OCOCH_3$), 3.9 (s, 3H, OCH_3), 6.9 (d, J=2 Hz, H-6), 7.2-7.6 (m, 4H).

KSI-4 (COMPOUND ISOLATED FROM LOWER BAND)

On crystallisation from methanol it gave a yellow crystalline compound (m.p. 310-11°, 45mg) which gave a pink colour with Mg- HCl, an intense yellow colour on exposure to ammonia vapours and a greenish colour with alcoholic $FeCl_3$ solution.

SPECTRAL DATA

U.V. (Methanol)

$\lambda_{max}^{Methanol}$ 256.0, 301 (inf), 370 nm.

$\lambda_{max}^{AlCl_3}$ 270, 302 (inf), 333, 456 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 265, 301, (inf), 359, 426 nm.

λ_{max}^{NaOAc} 273, 327 (inf), 390 nm.

$\lambda_{max}^{NaOAc+Boric\ acid}$ 261, 303 (inf), 388 nm.

Mass (m/e)

302 (M^+), 274

ACETYLATION OF KSI-4

The compound **KSI-4** (35 mg) was dissolved in pyridine (0.5 ml) and acetic anhydride (0.5 ml) was added to it. The contents were left overnight and worked up as usual. On crystallisation from methanol it gave a colourless crystalline compound (**KSI-4A**) (20 mg, m.p. 195-96°).

SPECTRAL DATA

^{1}HNMR (δ, $CDCl_3$)

2.4 (s, 12H, 4x$OCOCH_3$), 2.5 (s, 3H, $OCOCH_3$), 7.0 (H-6), 7.3 (d, J=8 Hz, H-5), 7.5 (H-8), 7.85 (m, 2H, H-2', H-6').

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CHAPTER - IV

DISCUSSION

STUDY OF THE FLOWERS OF *ACACIA LEUCOPHLOEA* (ROXB) WILLD. SYN. *MIMOSA LEUCOPHLOEA* (ROXB) (N.O.,MIMOSACEAE.)

VERNACULAR NAMES

Local Name - Safed Kikar, **Sanskrit**-Arimeda; Irimeda; Shvetabarbura, **Telgu**-Tella tumma, **Tamil**-Velvelam, **Bengali**-Safed babul, **Marathi**-Hewar, **Gujrati**-Haribaral, **Kannad**-Bilijali, **Malayalam**-Pattacharaya maram.

DISTRIBUTION

The plant is well represented in India particularly in Punjab, Central India, Andhra Pradesh, Karnataka and dry tracts of peninsular India. It is found through out Rajasthan but grows well in Jaisalmer, Bikaner, Jodhpur, Jaipur, Udaipur and Kota divisions. It occurs also in the dry zones of Burma[1,2].

BOTANICAL DESCRIPTION

It is a moderate sized deciduous tree, upto 3 metre in height. It is smaller in very dry localities and the stem is crooked and gnarled. It has strong, straight, white spines about 2.5 cm. in length. The branches are dense, leaves are bipinnate with spines having a cup shaped gland between each pair of pinnae. Flowers are in globose heads in long terminal tomentose panicles and are white to pale yellow in colour. Pods are black, ligulate, 10-20 cm long with ten to twenty seeds. The bark is light yellowish grey to nearly white and light red inside. The flowering takes place during July-November and fruiting from December to January[1,2].

MEDICINAL PROPERTIES AND USES

The plant has been described to be astringent demulcent and aphrodisiac. The bark is crushed and applied in bandage form over the traumatic ulcer. The speciality of this medicine is that even if the ulcer gets moistured by water, the curing process does not get retarded and no pus

gets formed. Its bark is reported to be bitter, cooling and used in bronchitis and biliousness. The gum is demulcent and used as an emulsifying agent. Leaves are used in syphilis[2-7].

The bark is used in the preparation of spirits from palm juice and sugar. It is reported to act as a clarifying and flavouring agent[2].

LITERATURE SURVEY

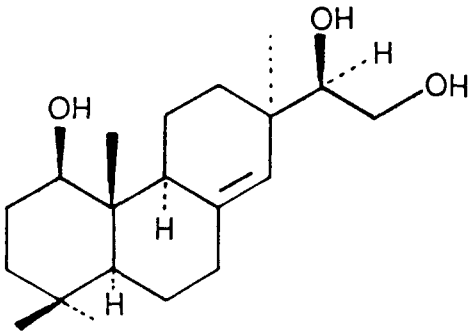
The total alcoholic extractives of the air dried and powdered heartwood were fractionated with petroleum ether, benzene, chloroform, ethyl acetate, acetone and methanol which were designated as fractions A to F respectively. The fractions B,C, E and F were found to possess antibacterial activity[8]. The plant has been reported to possess juvenomimetic activity producing adultoids in *Dysdercus cingulatus*[9].

Its flowers have been chemically examined by Mukerjee and Murty[10] in 1951 who isolated a crystalline flavonol, after acid hydrolysis of water soluble fraction. It was identified as myrecitin. The sugars present in the hydrolysate were isolated by preparing their osazones. Fractional crystallisation of the mixture of osazones from acetone gave glucosazone.

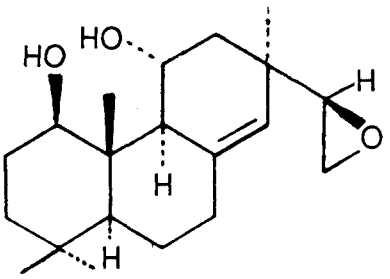
Kapoor *etal*[11] in 1971 have reported the following seed analysis: moisture 71%, protein 27.4%, pentosan 12.8%, water soluble mucilage 7.1% and protein in mucilage 29.0%. Tiwari and Srivastava[12] studied the oil composition of the seeds in 1981 and reported the following characteristics of the oil; saponification value 193.4-194.9%, iodine value 110.3-111.2, percentage of oil 12.61, acid value 1.0-1.1, ester value 193.8- 194.4%, specific gravity d_4^{40} = 1.108; viscosity E at 32°C-64.5, moisture-6.04%, ash content-1.04%, optical activity-no rotation in 2% solution of $CHCl_3$, soluble in organic solvents and showed charring with conc. H_2SO_4. The effect of temperature on viscosity of the oil was also studied. The soap prepared from its oil was found to be similar to the soaps prepared from edible oils.

Joshi and Sharma (1970)[13] reported that the benzene extracts of the stem bark on chromatographic separation over deactivated alumina, yielded n-hexacosanol, β-sitosterol and β-amyrin, while the petroleum-ether extract of the heartwood yielded-n-octacosanol and β-sitosterol.

From its root bark, two new pimar-8(14) ene-diterpenoids have been isolated by Bansal *etal*[14] and their structures established by chemical and spectroscopic methods as 1β, 15R, 16-trihydroxypimar-8(14)-ene (leucophleol) **(I)** and 15R, 16 epoxy-1β,11-dihydroxy pimer-8 (14)-ene (leucopheloxol) **(II)**.

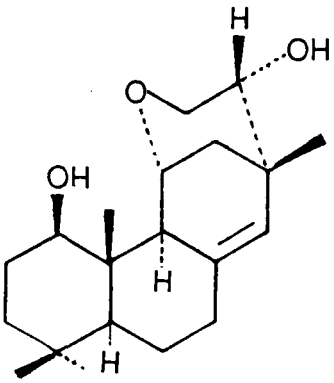


(I)



(II)

Perales *etal*[15] in 1980 isolated another new diterpenoid from the root bark whose structure was determined by X-ray diffraction analysis as 11 , 16-epoxy-1β, 15R dihydroxy isopimar-8 (14)-ene (Leucoxol) **(III)**.



(III)

Kedare and Tendolkar[16] (1952) have reported the results of the ash analysis of the heartwood of this plant; ash value-1.12%, Fe_2O_3 + Al_2O_3-0.46%, CaO-0-33.6%, MgO-14.31%. K_2O-20.54%, Na_2O-3.45%, SO_3-1.89%, C1-0.87% and P_2O_5-6.10%.

Srivastava and Agnihotri[8] (1984) isolated two compounds identified as octacosanol and (+) pinitol from the petroleum ether and ethyl acetate fractions of the heartwood of this plant.

Bhadoria and Gupta[17] (1981) have reported that its leaves and pods contain alarming quantities of hydrocyanic acid.

The composition of the air dried powdered seeds has been reported to have water 10.3%, ash-3.8%, cellulose 85.91%, fats 1.96%, pectin bodies 7.54%, water soluble products 2.16%, oil, 6.8% with following characteristics: refractive index at 30° 1.4871, acid value-6.3, iodine value-89.29, saponification value-197.96, Specific gravity-0.897. In addition to other fatty acids in seed oil the presence of coronaric acid was also established by physical and chemical methods.[18]

In view of the important medicinal properties of the plant, a detailed chemical investigation of its flowers collected from *A. leucophloea* trees growing in the campus of Hamdard Nagar, New Delhi was undertaken.

PRESENT WORK

The air dried flowers were exhaustively extracted with ethanol and the ethanolic concentrate was treated with petroleum ether to isolate petrol soluble products marked as **PET-S**. The residue left after petrol extraction was dissolved in water and filtered to give water soluble and water insoluble fractions marked as **W-S** and **W-INS** respectively. The water insoluble fraction (**W-INS**) in spite of repeated efforts did not yield pure products and hence further studies were abandoned.

COLUMN CHROMATOGRAPHY OF PETROLEUM ETHER SOLUBLE FRACTION (PET-S)

It was chromatographed over a column of silica gel and eluted with petroleum ether, benzene and ethyl acetate. As the ethyl acetate eluates gave very complex mixture of compounds which could not be purified, hence, further studies were not carried out.

ISOLATION AND CHARACTERISATION OF COMPOUND 'A'

The petroleum ether eluate was rechromatographed over a column of silica gel and eluted with petroleum ether. The first five fractions of 100 ml showed the same T.L.C. pattern and therefore combined together. On usual processing and crystallisation, it gave a colourless compound, m.p. 76°, marked as 'A'. It was identical with the product isolated earlier from its pods in our labs and characterised as **behenic ester** having the molecular formula: $C_{19}H_{39}COOC_{28}H_{57}$[19]. The I.R. spectra of the two compounds were superimposable.

ISOLATION AND CHARACTERISATION OF COMPOUND 'B'

The benzene eluate was evaporated to dryness to give a residue which gave a positive L.B. test. It was rechromatographed on a column of silica gel and eluted with petroleum ether, petrol-benzene mixture, benzene and acetone. The petrol-benzene eluate (3:1) gave a positive L.B. test. On usual processing and crystallisation, it gave a colourless compound, m.p. 137°, which on acetylation gave an acetyl derivative, m.p. 126°. On the basis of co-T.L.C., m.m.p. and co-IR these products were identified as **β-sitosterol** and β-sitosteryl acetate respectively.

As the petroleum ether eluate on evaporation to dryness yielded only a negligible quantity of the product and the petrol benezene mixture (1:1), benzene and acetone eluates gave complex inseparable mixtures, hence these chromatographic fractions were not investigated further.

STUDY OF ETHYL ACETATE EXTRACT FROM LIQUID-LIQUID EXTRACTION (ISOLATION AND CHARACTERISATION OF COMPOUND-C)

The residue left after evaporation of ethyl acetate was extracted with boiling dry acetone and filtered. The filtrate was concentrated to a small volume and left for crystallisation. A yellow crystalline compound m.p.280° was obtained which was found to be pure. It gave positive tests for flavonoids and a positive Molisch test for glycosides.

The U.V. spectrum of the compound showed two maxima at 257(band II) and 363 (Band I) with an inflexion at 289 nm typical of 3-oxygenated flavonoids. The shift of band I to 423 nm on addition of $AlCl_3$ which moved to 407 nm on addition of HCl is consistent with a 5-hydroxy flavonol

with the possibility of the presence of a catecholic unit in the side phenyl. This is further supported by approximately 19 nm. bathochromic shift shown by band I in presence of sodium acetate/boric acid typical of 3',4'-dihydroxy system. Sodium acetate alone shifted band II from 257 nm to 271 nm. indicating the presence of free 7-OH group. These data indicate the presence of 3,5,7,3',4'-pentahydroxy system possibly carrying sugar moiety at 3 position.

The above glycoside on hydrolysis by Killiani's mixture gave an aglycone which was identified as quercetin and the sugar was identified as glucose and hence the glycoside was characterised as **quercetin-3-glucoside**.

STUDY OF BUTANOL EXTRACT FROM LIQUID-LIQUID EXTRACTION (ISOLATION AND CHARACTERISATION OF COMPOUND 'D'

The butanolic extract was removed and dried over anhydrous sodium sulphate. The inorganic salt was filtered off and filtrate was left at room temperature, when a light brown crystalline compound was obtained which on re-crystallisation with aqueous ethanol gave a colourless crystalline compund 'D'. It was identified as **mannitol** by m.m.p.,co-paper chromatography and superimposable I.R. spectra. It gave an acetate, m.p. 111° which was identical with mannitol hexaacetate.

EXPERIMENTAL

STUDY OF THE FLOWERS OF *ACACIA LEUCOPHLOEA* (ROXB) WILLD. SYN.,*MIMOSA LEUCOPHLOEA* (ROXB) (N.O. MIMOSACEAE.)

Fresh flowers were collected during the month of September from the campus of Hamdard Nagar, New Delhi and dried under shade.

EXTRACTION

The air dried flowers (1 kg) were exhaustively extracted (five times) with hot ethanol (95%). All the ethanolic extracts were combined together. Recovery of solvent left a dark brown viscous mass (80 gm) which was extracted with petroleum ether (60-80°) to give petrol soluble product (**PET-S**). The residue left after petrol extraction was taken up in water (700 ml) and filtered to give water soluble (**W-S**) and water insoluble (**W-INS**) (5 gm) fractions. The water insoluble fraction inspite of repeated efforts could not be purified.

STUDY OF PETROL SOLUBLE PRODUCT (PET-S)

Recovery of petroleum ether left a light brown waxy mass (20 gm) which gave a green colour on L.B. test, indicating the presence of steroidal compounds.

COLUMN CHROMATOGRAPHY OF PET-S

The petrol soluble product (20 gm) was subjected to column chromatography over silica gel (60-120 mesh, 200 gms, diameter of the column 2.5 cm.). The column was eluted with light petroleum ether (1.7 lit.), benzene (1.5 lit.) and ethyl acetate (1.0 lit.).

ISOLATION OF COMPOUND 'A' FROM PETROLEUM ETHER ELUATE

The petroleum ether eluate on recovery of solvent left a light brown waxy mass which crystallised from petrol to give a dull white crystalline compound (500 mg). A T.L.C. examination in light petroleum ether: benzene: (1:3) showed it to be mixture of two components (one major and

one minor, plate developed in iodine chamber). In order to purify, it was rechromatographed on a column of silica gel (60-120 mesh; 10 gm). The column was eluted with petroleum ether and ten fractions of 100 ml each were collected. The first five fractions showed the same T.L.C. pattern and were therefore combined together and concentrated to a small volume. On leaving at room temperature, a T.L.C. pure colourless crystalline compound (m.p. 76°, 100 mg) was obtained which was identified as behenic ester.

ISOLATION OF THE COMPOUND 'B' FROM BENZENE ELUATE

Benzene eluate on evaporation to dryness gave a residue (3.0 gm) which gave a green colour on L.B. test. It was rechromatographed on a column of silica gel (60-120 mesh; 60 gm, column width 1.0 cm) and eluted with petroleum ether (500 ml), petrol-benzene mixtures (3:1 and 1:1, 400 ml each), benzene (500 ml)and finally with acetone (200 ml). The petrol-benzene (3:1) fraction (400 ml) gave a green colour by L.B. test. It was evaporated to dryness and crystallised with methanol to give a dull white compound which on repeated crystallisation with methanol gave a colourless compound (m.p. 137°, 100 mg), m.m.p. with an authentic sample of β-sitosterol (136-137°). The IR spectra of the compound and β-sitosterol were also superimposable.

ACETYLATION OF COMPOUND B

The compound (70 mg) was dissolved in pyridine (1.0 ml) and acetic anhydride (1.0 ml) was added to it. The contents were heated on a boiling water bath under anhydrous conditions for two hours. On usual work up followed by crystallisation from methanol gave a colourless compound (m.p. 126°, 35 mg), m.m.p. with β-sitosterol acetate 124-25°. It gave a positive L.B. test (green colour) for steroids. On co-T.L.C. examination with β-sitosterol acetate, it was found to be identical. The IR spectrum of the compound was superimposable with that of β-sitosterol acetate.

STUDY OF WATER SOLUBLE FRACTION (W-S)

The water soluble fraction was transferred to a liquid-liquid extractor and subjected to liquid-liquid extraction with petrol (60-80°), solvent ether, ethyl acetate and butanol. The petrol (60-80°) and solvent ether extracts did not yield any appreciable quantity of products for further study.

STUDY OF ETHYL ACETATE EXTRACT FROM LIQUID-LIQUID EXTRACTION (ISOLATION OF COMPOUND 'C')

The ethyl acetate layer was dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The residue was extracted with boiling dry acetone and filtered. The filtrate was concentrated to a small volume and left for crystallisation at room temperature. A yellow crystalline compound, m.p. 280° (10 mg) was obtained which on T.L.C. examination in chloroform: methanol: water (70:27:3.6) and paper chromatographic examination in butanol, acetic acid, water (4:1:5) was found to be a single entity. It gave a red colour with Mg/HCl, a green colour with $FeCl_3$ and an intense yellow colour on exposure to ammonia vapours and showed a brown flouresence under U.V. light. It gave a positive Molisch test.

U.V. (METHANOL)

λ_{max}^{MeOH} 257, 289 (inf), 363 nm.

$\lambda_{max}^{AlCl_3}$ 270, 313, 367, 423 nm.

$\lambda_{max}^{AlCl_3 + HCl}$ 270, 310, 363, 407 nm.

λ_{max}^{NaOAc} 271, 323, 388, 396 nm.

$\lambda_{max}^{NaOAc/H_3BO_3}$ 258, 304, 382 nm.

HYDROLYSIS OF COMPOUND 'C'

The compound (7.0 mg) was hydrolysed by killiani mixture (1.0 ml) by heating in a test tube for 2 hours. The contents were then diluted with water (10 ml) and extracted with solvent ether five times (5x10 ml) in order to separate the aglycone. All the ethereal extracts were combined together

and washed with water. The ethereal layer was dried over sodium sulphate. Recovery of solvent left a very small dark brown residue which by co-T.L.C. examination with quercetin was found to be identical with it.

IDENTIFICATION OF SUGAR

The aqueous layer left after extraction with ether (Killiani's hydrolysis) was evaporated to dryness in a china dish on a water bath and dissolved in a few drops of distilled water. It gave a red precipitate with Fehling's solution, showing the presence of reducing sugars. On co-chromatography with glucose (as described on page 38) it was identified as glucose.

STUDY OF BUTANOL EXTRACT FROM LIQUID-LIQUID EXTRACTION (ISOLATION OF COMPOUND 'D')

The butanol layer was removed and dried over anhydrous sodium sulphate. The inorganic salt was filtered off and the filtrate after leaving at room temperature for one month a light brown crystalline compound (m.p. 163-64°) was obtained which was separated and recrystallised from aqueous ethanol (ethanol: water: 3:1) to give a colourless crystalline compound 'D' (m.p. 165°, 200 mg). It was sweet in taste and did not reduce Fehling's solution. It gave positive test for polyhydric alcohols (a few mg of the substance + 1 ml of 1% borax solution containing a two drops of phenolphthalein → the pink colour disappeared, which reappeared on warming and vanished again on cooling). It was identified as mannitol by m.m.p., descending co-paper chromatography (ethyl acetate, pyridine, water: 7,5,2) and co-I.R. spectra.

ACETYLATION OF THE ABOVE COMPOUND

The compound (100 mg) was refluxed with acetic anhydride (3 ml) and fused sodium acetate (200 mg) for two hours. On usual work up followed by crystallisation with dichloromethane-petroleum ether (60-80°) mixture gave a colourless acetyl derivative (100 mg) m.p. and m.m.p. 111° with an authentic sample of mannitol hexaacetate. The I.R. spectrum of the acetate and that of mannitol hexaacetate were superimposable.

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CHAPTER - V

DISCUSSION

STUDY OF THE FRESH FLOWERS OF *DODONAEA VISCOSA* LINN (N.O. SAPINDACEAE)

VERNACULAR NAMES

Hindi-Sinatha, Aliar; **Telgu**-Bandedu **Tamil**-Velari; **Kannad**-Bandara; **Malayalam**-Unnataruvi; **Punjabi**-Benmenu; **Bihar**-Mehndi; **Orissa**-Mohra; **Bombay**-Jakhmi.

DISTRIBUTION

It is found almost throughout India. In Western Himalayas it is found gregariously as an under growth in the forests of *Pinus roxburghii* Sarg. and in dry miscellaneous forests ascending to a height of 6,500 ft. It is also found in the dried regions of South India upto an altitude of about 8,000 ft.[1]

BOTANICAL DESCRIPTION

It is an evergreen shrub or a small tree and often a climber. Its leaves are shining and flowers are greenish yellow. Capsules are thin, winged and yellowish brown and seeds are black. The flowers are found during Aug.-Feb. while fruiting takes place from Feb. to March.[2]

MEDICINAL IMPORTANCE AND USES

The leaves are used in the treatment of wounds, swelling and burns. They are also used as febrifuge and sudorific in gout and rheumatism. An embrocation of the leaves is applied to sprains and bruises. In Peru, the leaves are chewed as a stimulant and are used as an adulterant of coca leaves. The bark is employed in astringent baths and fomentation. In Punjab the leaves are applied as poultice to snake bites and their juice given internally. The seeds are said to be edible and the fruits were once used as a substitute for true hops (*Humulus lupulus* Linn) and in making yeast and beer.[1,3] Isorhamnetin 3- rhamnosylgalactoside isolated from its aerial parts showed 15% blood sugar lowering effect[4]. The saponin mixture of dodonoside-A and B isolated from the seeds showed antiexudative, phagocytosis -enhancing and molluscicidal activity.[5] The growth of *Staphylococcus albus* was very effectively inhibited by the essential oils of the seeds[6].

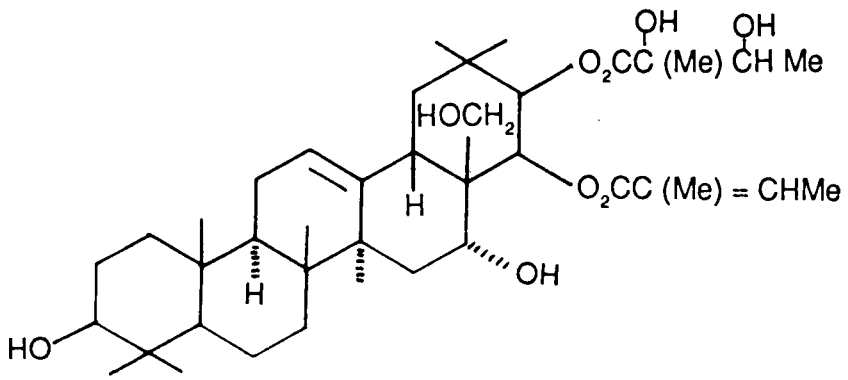
LITERATURE SURVEY

A survey of the literature showed that almost all parts of the plant contained flavonoids and saponins.

The bark and leaves have been reported to contain tannins to an extent of 33% and 10% respectively[7]. Sastry and Nayudamma[8] isolated a leucocyanidin, as a pale brown powder, m.p. 235° from the bark and characterised it as a new isomer of 5,7,3',4'-tetrahydroxy flavan 3,4-diol but did not characterise it completely. They also identified glucose, shikimic acid and chlorogenic acid by paper chromatography.

Recently Ahmad and Khan[9] reported the presence of isorhamnetin and quercetin in its root bark.

From the saponin isolated from the stem bark, two sapogenins R_1-barrigenol, jegosapogenol and two novel prosapogenins, R_1-barrigenol 21, 22-diangelate and jegosapogenol 21-(2,3-dihydroxy-2 methyl butyryl) 22-angelate (I) were isolated by Zeza *etal* in 1985[10]. Their structures were elucidated by spectroscopy.



(I)

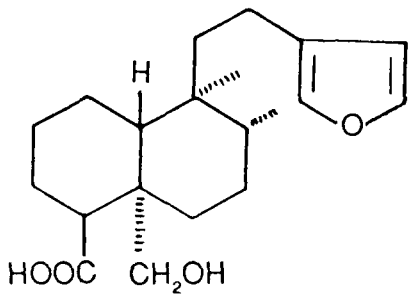
The saponin from the stem on acid hydrolysis gave stigmasterol, 21, 22-diangeloyl barringtogenol C, 21, 22- diangeloyl-R_1-barringenol, 21-angeloyl-R_1 barringenol and cleomiscosin A.[11]

From the leaves and pods, isorhamnetin 3-O-rutinoside, quercetin-3-O galactoside and quercetin 3-O rutinoside have been isolated by Nair and Subramanian[12].

Paris and Nothis[13] reported that the leaves contained quercitol, kaempferol, arabinose, glucose and rhamnose which suggested the presence of rutoside or avicularoside (quercitol-3-arabinoside). Chlorogenic acid and caffeic acid were present but flavanones were absent.

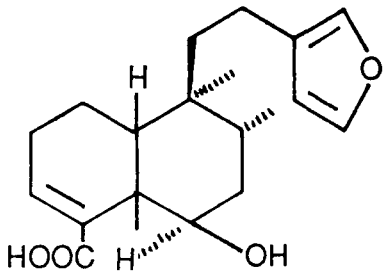
Rao, K.V. in 1962 isolated three compounds from the leaves, which were identified as β-sitosterol, stigmasterol and isorhamnetin[14].

Kotake and Kiwata[15] isolated hetriacontane and a diterpenoid hautriwaic acid from the resin of the leaves and characterised it partially as monohydroxy carboxylic acid. Later on it was characterised as (**II**) by Hong-Yen Hsu *etal*[16].



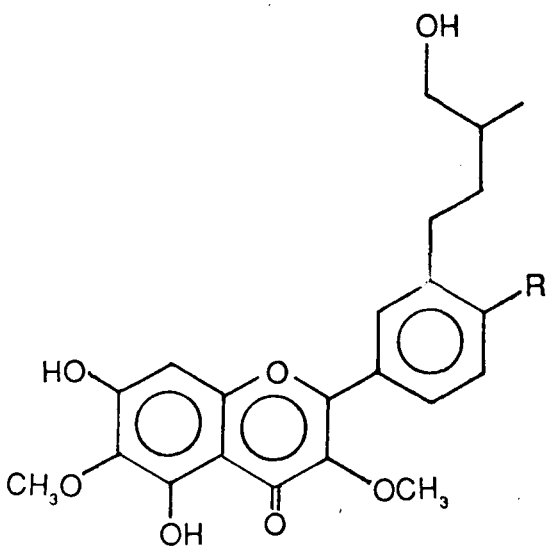
(II)

From the aerial parts of plant, β-sitosterol, β-sitosterol-3-β-D-glucoside, stigmasterol, inositol monomethyl ether and dodonic acid, a new diterpenoidic acid of ent-clerodane series, were isolated by Sachdev and Kulshreshtha[17]. The structure and stereochemistry of dodonic acid (**III**) have been established by chemical and spectroscopic methods.



(III)

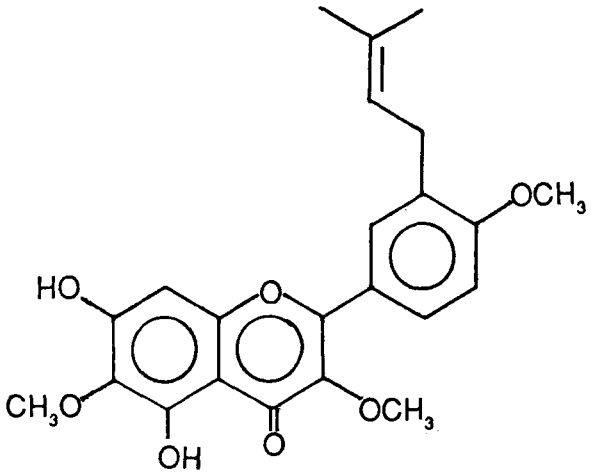
Aliarin, a new flavonoid, was isolated from the aerial parts of this plant by Sachdev and Kulshreshtha[18]. Its structure was established as 5,7,4'-trihydroxy-3'-(3-hydroxy methyl butyl)-3,6-dimethoxy flavone (**IV**) on the basis of chemical and spectroscopic evidences. Further investigation of the aerial parts of this plant[19] afforded a new flavonoid having an isoprenoid side chain along with seven known flavonoids, 5-hydroxy-3,6,7,4'-tetramethoxy flavone, pinocembrin, santin, penduletin, 5, 7, 4'-trihydroxy-3,6-dimethoxyflavone and isorhamnetin 3-rhamnosyl galactoside. The structure of the new flavonoid was elucidated as 5,7-dihydroxy-3'-(3-hydroxymethyl butyl)-3, 6, 4'-trimethoxy flavone (**V**).



(**IV**) R = OH

(**V**) R = OCH_3

From the aerial parts one more new prenylated flavonoid named as viscosol was isolated by Sachdev and Kulshreshtha[20] in 1986. The structure of viscosol was established as 3-(γ,γ-dimethylallyl) 5,7-dihydroxy-3,6,4'-trimethoxy flavone (**VI**) on the basis of spectral data as well as by chemical studies.



(VI)

Dominguez *etal* in 1980[21] isolated 5,7-dihydroxy-3,6,4'-trimethoxy flavone and β-sitosterol from the aerial parts of *D. Viscosa*, variety-angustifolia Jacg.

Parihar and Dutt isolated a glucoside dodonin and a fixed oil from the seeds in 3.42% and 20.27% yield respectively. Dodonin on acid hydrolysis gave dodogenin with the molecular formula $C_{23}H_{36}O_8$ having a cyclo-penteno-phenanthrene nucleus. However, they did not elucidate the structures of dodonin and dodogenin.

The composition of the seed oil has been determined by Gupta [23,24] and the essential oil constituents of the seeds have been determined by Mekkawi and Mossa[25].

Except for the isolation of 5,4'-dihydroxy-3,7-dimethoxy flavone and 5-hydroxy 3,7,4'-trimethoxy flavone from its flowers by Dreyer in 1987[26], no work seems to have been done on the flowers and hence the present studies were undertaken.

PRESENT WORK

EXTRACTION

The fresh flowers were collected during the months of March/ April from the campus of Hamdard Nagar, New Delhi. The flowers on exhaustive extraction with ethanol under refluxing conditions and recovery of solvent left a syrupy mass which was successively extracted with petroleum ether (60-80°), chloroform, acetone and methanol.

STUDY OF THE CHLOROFORM EXTRACT

The chloroform extract gave positive tests for the presence of flavonoids. It was chromatographed on a column of silica gel. The fraction eluted by benzene: ethyl acetate (4:1) gave a yellow compound, m.p. 200-201°. A T.L.C. examination showed it to be a mixture of two components, which were separated by preparative T.L.C. into two pure fractions **A** and **A-1**. As the quantity of **A-1** was inappreciable, hence, further studies were not carried out. The compound **'A'** on crystallisation with methanol gave bright yellow needles, m.p. 203-204°.

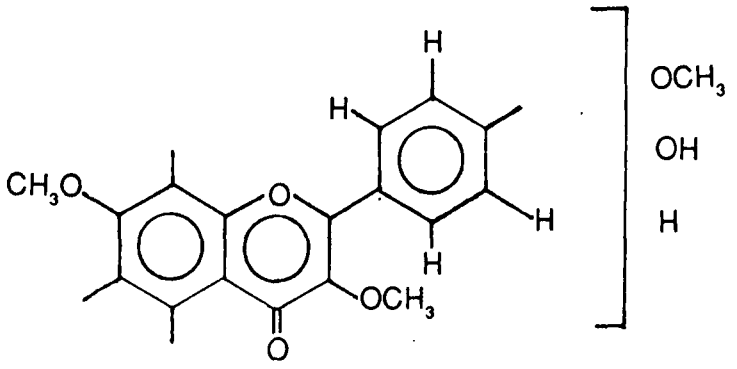
CHARACTERISATION OF COMPOUND 'A' M.P. 203-204°

It gave usual tests for flavonoids. On acetylation with pyridine/acetic anhydride, it gave an acetate, m.p. 172-73°. In the U.V. spectrum it showed two maxima at 271.5 (band II) and 339.6 nm (Band I). Addition of sodium acetate did not show any change in the position of band II which suggested either the absence of hydroxyl group at 7-position or its protection as-OCH_3 group. However, the location of band II at 271.5 nm. suggested oxygenation at 7 position. Coupled with the above information the conclusion may be drawn that there is an OCH_3 group at 7-position. There was no change in the spectrum by the addition of boric acid which suggested absence of free catecholic unit in the side phenyl ring.

Addition of $AlCl_3$ shifted band I to 363.1 nm. which suggested the presence of a free OH group at 5 position. The location of band I at 339.6 nm. in methanol suggested a protected hydroxyl, possibly in the form of a -OCH_3 group at position 3. Addition of HCl to $AlCl_3$ containing solution did not produce any appreciable change in the spectrum which also supported above findings.

NMR spectrum of the parent compound in $DMSOd_6$ showed three methoxyls by three singlets δ 3.85, δ 3.9 and δ 4.1, each integrating to three protons, suggesting that the compound is a trimethyl ether. In the aromatic region there was a singlet for one proton at δ 6.78 and an A_2 B_2 pattern at δ 7.0 and δ 8.0 as two doublets with (J = 8 Hz). The last pattern suggested a 4' substituted side phenyl, the lone aromatic proton indicated by a signal at δ 6.78 could either be due to H-3 of the pyrone ring or from the lone proton of a trioxygenated A-ring of the flavonoid moeity.

The NMR spectrum of the acetate (**A-A**) showed two acetoxyl signals at δ 2.3 and δ 2.5 besides the three methoxyl signals at δ 3.75, δ 3.8 and δ 3.9. The aromatic region was very similar to the spectrum of the parent compound, the lone proton signal being located at δ 6.8 and the A_2 B_2 system at δ 7.2 and δ 8.05 coupled with this information it could be inferred that the compound is a trimethyl ether of pentahydroxy flavone. Together with the U.V. spectral information, this points out to the following possibilities.



Of these a further distinction can be made on the basis of the acetylation has shifted the high field A_2 B_2 doublet by 0.2 ppm. downfield. This could mean that the-OH is located at 4' position in the parent compound. This conclusion is only as the solvents used for the NMR of the parent compound ($DMSOd_6$) and its acetate ($CDCl_3$) are different. This suggests that the compound could be 3,6,7 trimethoxy 4', 5-dihydroxy flavone or 3,7,8-trimethoxy 4', 5-dihydroxy flavone. The first structure corresponds to penduletin and the second structure corresponds to herbacetin 3,7,8 trimethyl ether[27]. Comparison of the reported properties of the above two compounds with our compound led to the conclusion that our compound is **penduletin.**

Similar conclusions were drawn by ^{13}CNMR spectrum of the parent compound. The spectrum was taken in the mixture of $DMSOd_6/CDCl_3$. It showed 16 signals of which three showed up as quartets, three as doublets and rest as singlets in the off-resonance decoupled spectrum. The following table gives the chemical shifts. The off-resonance pattern and the assignments are made on usual considerations of chemical shifts, additivety parameters and literature data.

1.	δ 56.3	Quartet	$-OCH_3$
2.	δ 59.5	"	$-OCH_3$
3.	δ 59.9	"	$-OCH_3$
4.	δ 99.0	Doublet	C-8
5.	δ 105.8	Singlet	C-10
6.	δ 115.8	Doublet	C-3',C-5'
7.	δ 119.9	Singlet	C-1'
8.	δ 130.1	Doublet	C-2', C-6'
9.	δ 131.7	Singlet	C-6
10.	δ 137.5	"	C-3
11.	δ 151.8	"	C-5 or C-9
12.	δ 151.9	"	C-9 or C-5
13.	δ 156.2	"	C-2
14.	δ 158.5	"	C-7
15.	δ 161.3	"	C-4'
16.	δ 178.2	"	>C=O

Obviously the first three high field signals are due to the three methoxyl groups. The signal at δ 99.0 is due to a protonated aromatic carbon, the chemical shifts typical of the C-6 or C-8 of a 5,7 dioxygenated flavonoid. The signals at δ 115.8 and δ 130.1 showed almost double intensity compared to the signal at δ 99.0 and hence each could arise from two carbons. This could be easily assigned to C-3', 5' and C-2', 6' because of the local symmetry of a 4' substituted side phenyl. This is also reflected in the reduction of the total number of signals from 18 to 16. Of the remaining signals, the two signals at δ 105.8 and δ 119.9 are due to non-protonated, non-oxygenated carbons. In the flavonoidal skeleton, these can be assigned to C-10 and C-1' respectively from chemical shift consideration. This leaves signals in the range of δ 131.7 to δ 178.2, the last signal being the carbonyl carbon. The remaining seven signals are obviously due to five oxygen bearing aromatic carbons and C-2 and C-9 carbon atoms. Literature data lead to the assignment of the

signals at δ 156.2 and δ 137.5 to C-2 and C-3 carbons. Study of the ^{13}C NMR spectra of 5,6,7 and 5,7,8 trioxygenated flavonoids indicates that C-6 in the former and C-8 in the latter are doubly shielded by two ortho oxygen substituents and hence resonate at comparatively higher fields. This leads to the assignment of the signal at 131.7 to C-6 of the remaining four signals namely due to C-5, C-7, C-9 and C-4', only the signal due to C-4' can be expected at the lowest field as it is not subjected to any shielding influence and hence the signal at δ 161.3 can be assigned to C-4'. Remaining assignments are shown in the **Table** (page 103).

A study of the mass spectra of the parent compound and its acetate also favoured the above findings. The mass spectrum of the compound showed the molecular ion peak at m/e 344 consistent with the molecular formula $C_{18}H_{16}O_7$. It showed a methyl loss to give a base peak at m/e 329. Generally this abundant M-15 peak, is indicative of a-OCH_3 group at C-6 or C-8. This is followed by an expulsion of CO molecule to give a peak at m/e 301 or a loss of 42 mass units to give a peak at m/e 287. The loss of CO is also a known fragmentation in flavonoids[28]. The loss of 42 mass units is known to occur from a C-3 methoxyl substituent.[28] In the low mass region the two prominent peaks at m/e 181 and m/e 153 are accountable by RDA fragmentation from M-15 peak leading to the A-ring ion (m/e 181) followed by loss of CO (m/e 153). Another peak at m/e 121 can be ascribed to p-hydroxy benzoyl cation, which is indicative of 4' hydroxyl group (**Chart-I**).

The mass spectrum of the acetate also leads to very much the same conclusions. (**Chart-II**).

STUDY OF THE ACETONE EXTRACT

The residue obtained after distillation of acetone was dissolved in water and filtered. The filtrate on hydrolysis gave an aglycone, black in colour, which was filtered, washed with water, dried and extracted with solvent ether. The ethereal concentrate was chromatographed on a column of silica gel. The benzene: ethyl acetate (3:1) eluate on T.L.C. examination showed two brown spots under U.V. light which were separated by preparative T.L.C. on silica gel G plates, to give compound '**B**', m.p. 305-306° (upper band) and compound **C**, m.p. 313-14° (lower band).

CHARACTERISATION OF COMPOUND 'B' (M.P. 305-306°)

It gave positive tests for flavonoid. On acetylation in the usual way, it gave an acetyl derivative, m.p. 202-203° (**B-A**).

CHART-I

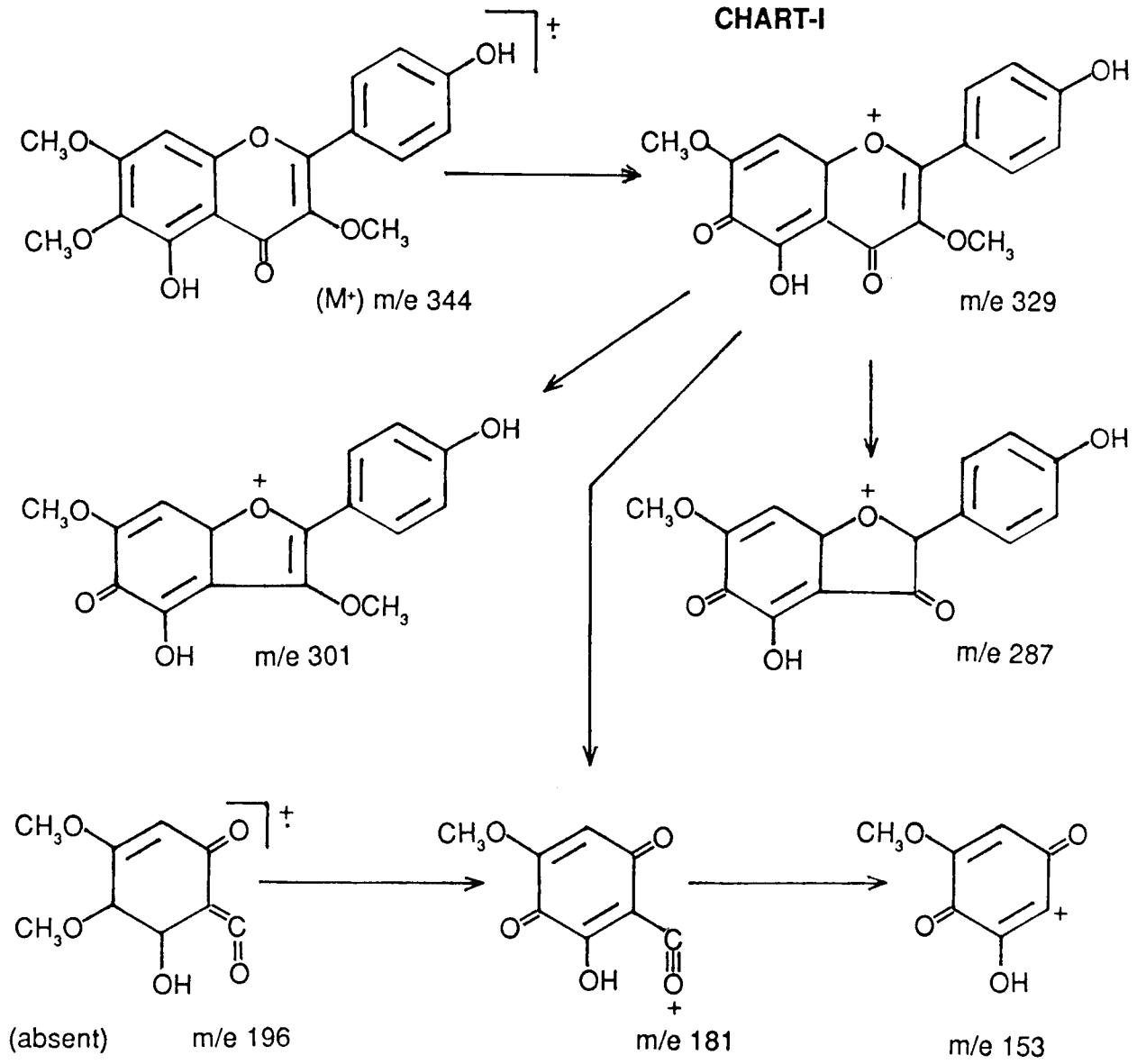
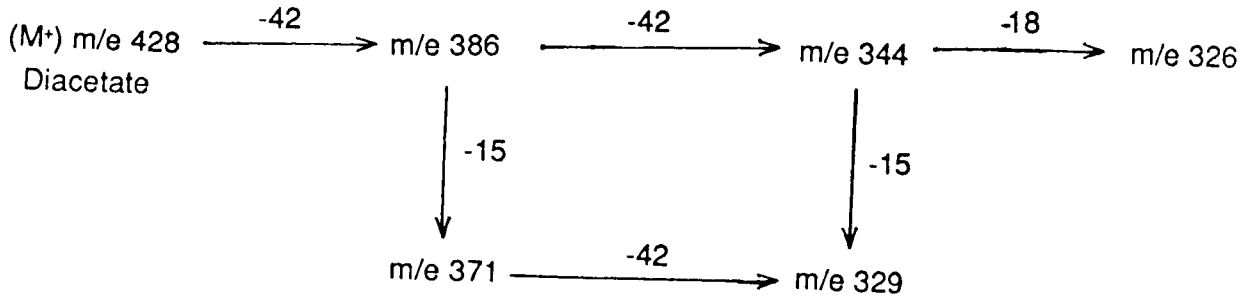


CHART-II



The U.V. spectrum of the parent compound (**B**) showed two maxima at 255.2 nm (band II) and 371.2 nm (band I) with two inflexions at 267.38 nm and 326.75 nm. On addition of $AlCl_3$, $AlCl_3$/ HCl, NaOAc and NaOAc/H_3BO_3 the behaviour of the shifts in the maxima of the spectra was the same as observed in the case of isorhamnetin (Page 75).

Further proof for its identity as isorhamnetin was obtained by NMR spectral studies of its acetate. The ^{1}HNMR spectrum of the acetate showed four phenolic acetoxyls at δ 2.3-δ 2.5 and one methoxyl at δ 3.9. In the aromatic region there was one proton metacoupled doublet at δ 6.96 (J=2 Hz) characteristic of H-6 of a 5,7 dihydroxy flavonoid. There was a multiplet for two protons centred at δ 7.4 partly overlapping with $CHCl_3$ signal and a two proton multiplet centred around δ 8.0. There was no sharp signal for H-3 in the expected region. Thus these data are consistent with the compound being mono methyl ether of quercetin i.e. isorhamnetin.

Mass spectrum of the compound showed molecular ion peak at m/e 316 corresponding to mol. formula $C_{16}H_{12}O_7$. This showed loss of CO, $CO+CH_3$ to give peak at m/e 288 and 273. These findings are consistent with the formulation of the compound as **isorhamnetin**. The mass fragmentation pattern of the compound shown in the **chart** (Page 76).

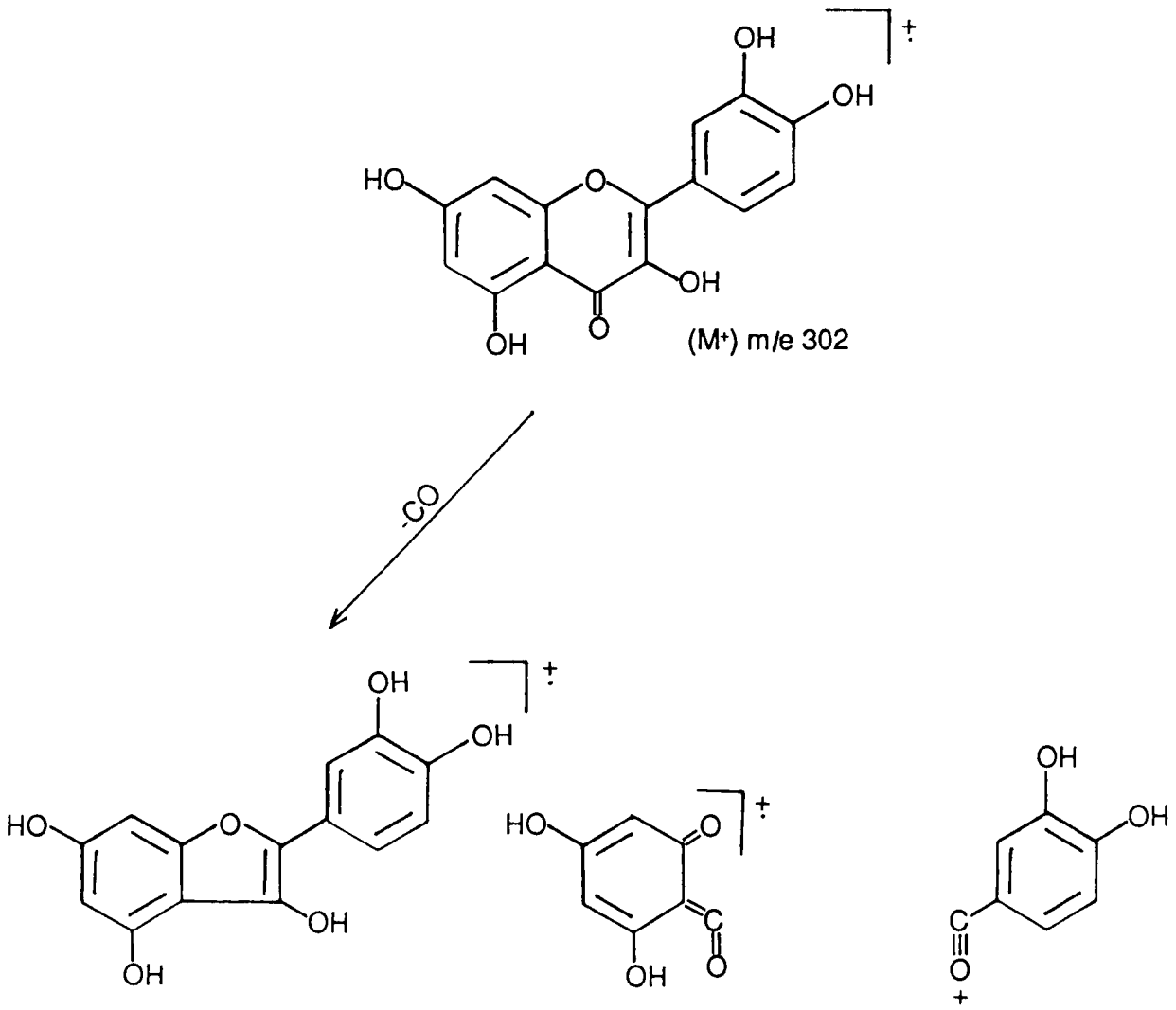
CHARACTERISATION OF COMPOUND 'C' (M.P. 313-14°)

It gave positive tests for flavonoid. The U.V. spectrum of the compound showed two maxima at 256 (band II) and 370.2 nm (band I) with an inflexion at 301 nm. On addition of $AlCl_3$, $AlCl_3$/HCl, NaOAc, NaOAc/H_3BO_3 the behaviour of the shift in the maxima of the spectra was the same as observed in the case of quercetin (Page 77).

Further proof for its identity as quercetin was obtained by mass spectral studies.

In the mass spectrum it showed the following RDA fragmentation pattern which was characteristic for quercetin (**Chart-III**). The molecular ion peak was observed at 302 and a peak at m/e 274 was for ion(a), the peak at m/e 152 was for ion(b) and the peak at m/e 137 was for ion(c). These data accounted for the structure of the compound as **quercetin**.

CHART-III



(a) m/e 274

(b) m/e 152

(c) m/e 137

STUDY OF METHANOL SOLUBLE FRACTION AND ISOLATION OF COMPOUNDS D, E & F.

The residue left after recovery of methanol was dissolved in water and filtered. The filtrate gave positive tests for the presence of flavonoids and saponins.

HYDROLYSIS OF THE FILTRATE

The filtrate on hydrolysis with sulphuric acid gave a black precipitate which was filtered, washed with water and dried. It was successively extracted with chloroform and solvent ether.

STUDY OF THE CHLOROFORM EXTRACT OF THE AGLYCONE

Recovery of solvent gave a semi-solid brown residue which on T.L.C. examination was found to be a mixture. Inspite of repeated attempt it could not be crystallised and was converted into its acetyl derivative in the usual way. The acetate on T.L.C. examination was found to be a mixture.

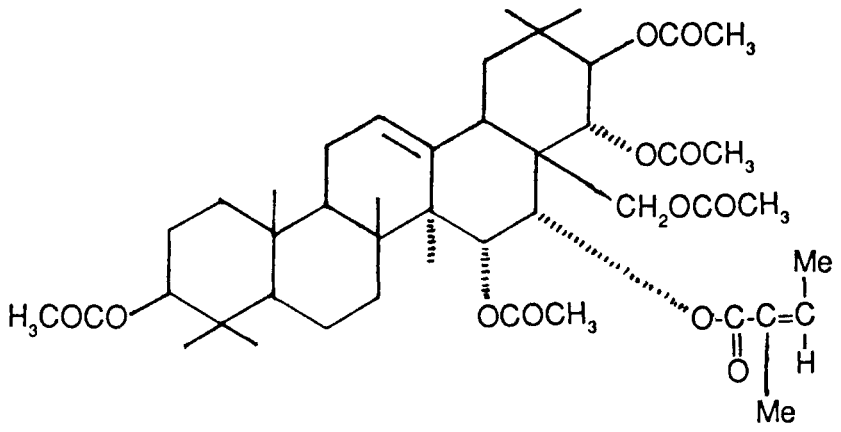
ISOLATION AND CHARACTERISATION OF THE COMPOUND 'D'

The crude acetyl derivative on fractional crystallisation with methanol containing a few drops of chloroform gave a colourless compound m.p. 202-03° which on deacetylation with methanolic HCl gave a yellow compound m.p. 302°. It gave usual tests for flavonoids and was designated as compound **'D'**. On the basis of the NMR data of the acetyl derivative which was similar to that noted on (page 106), it was identified as **isorhamnetin tetraacetate**. These findings were further supported by the U.V. spectral data with shifts of the deacetylated product. m.p. 302° which were in agreement with the U.V. data reported for isorhamnetin (Page 75).

STUDY OF THE MOTHER LIQUOR OF ISORHAMNETIN ACETATE, ISOLATION OF COMPOUND 'E'

The mother liquor left after removal of isorhamnetin acetate (compound **D**) was heavily charcoaled in order to remove flavonoidal constituents. It was concentrated to a small volume and left at room temperature, which gave a colourless compound, m.p. 283-85°. It gave a positive L.B.

test showing it to be triterpenic in nature. It was found to be identical with the product isolated earlier from its seeds in our lab[29] and characterised as **doviscogenin acetate** on the basis of co-T.L.C., m.m.p. and co-IR spectrum.



STUDY OF THE ETHER SOLUBLE FRACTION OF THE AGLYCONE LEFT AFTER CHLOROFORM EXTRACTION, ISOLATION OF COMPOUND F

Recovery of ether left a brown solid which on several crystallisations from methanol gave a yellow compound, m.p. 304- 305°. It gave positive tests for flavonoids. On the basis of U.V. spectral data it was identified as **isorhamnetin**. On acetylation in the usual way, it gave an acetyl derivative, m.p. 202-03° which was also identical with isorhamnetin tetraacetate (m.p., m.m.p. and co-T.L.C.).

EXPERIMENTAL

STUDY OF THE FLOWERS OF *DODONAEA VISCOSA* LINN. (N.O. SAPINDACEAE)

EXTRACTION

The fresh flowers (900 gms) were collected during the month of March/April from the campus of Hamdard Nagar and exhaustively extracted with hot ethanol (95%) seven times (till the last extract became almost colourless). All the ethanolic extracts were combined together and the solvent was recovered under reduced pressure. The residue (65 gms) was successively extracted with petroleum ether (60-80°), chloroform, acetone and methanol.

STUDY OF CHLOROFORM EXTRACT

Recovery of solvent left a dark green solid (5.0 gms) which gave a pink colour with Mg/HCl and an intense yellow colour with ammonia vapours indicating the presence of flavonoids. On T.L.C. examination in $CHCl_3$: MeOH (95:5) it was found to be a complex mixture.

PURIFICATION OF THE ABOVE CHLOROFORM CONCENTRATE BY COLUMN CHROMATOGRAPHY

It was subjected to column chromatography on silica gel (120 gm, 60-120 mesh, column width 2.5 cm.). The column was successively eluted with petroleum ether (60-80°) (500 ml), benzene (500 ml), benzene: ethyl acetate mixture; 4:1 (1.5 lit) and finally with ethyl acetate (500 ml). The ethyl acetate - benzene mixture eluate gave a pink colour with Mg/HCl and a yellow colour with ammonia vapours. It was evaporated to dryness and crystallised with methanol to give a bright yellow crystalline compound (m.p. 200-201°, 300 mg). On T.L.C. examination in chloroform: methanol (95:5) solvent system, it was found to be a mixture of two components with close Rf values (0.73 & 0.75). For future reference, the upper spot was marked **'A'** (Rf. = 0.75) and the lower spot as **'A-1'** (Rf. = 0.73).

SEPARATION OF COMPOUNDS 'A' AND A-1 BY PREPARATIVE T.L.C.

The above mixture was separated by preparative T.L.C. on silica gel G plates using chloroform: methanol (95:5) as solvent system. About 125 plates (size 20x20 cm.) were run. The

eluted plates were allowed to dry in the air. The two bands were marked with the help of U.V. light and carefully scrapped and transferred into two separate small columns. On being eluted with methanol, and evaporation of the solvent, the two compounds **'A'** and **'A-1'** were obtained. Compound **'A'** on crystallisation with methanol gave bright yellow needles, m.p. 203-204° (175 mg) which gave a pink colour with Mg - HCl, an intense yellow colour with NH_3 vapours and a green colour with alcoholic $FeCl_3$ solution. As the quantity of compound **'A-1'** was highly inappreciable, further studies were not carried out.

SPECTRAL DATA (COMPOUND 'A')

U.V. (METHANOL)

λ_{max}^{MeOH} 271.5, 339.6 nm.

λ_{max}^{NaOAc} 271.5, 346.6 nm.

$\lambda_{max}^{NaOAc+H_3BO_3}$ 271.5, 341.7 nm.

$\lambda_{max}^{AlCl_3}$ 282.3, 303.4(inf), 363.1 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 282.8, 301(inf), 358.3 nm.

^{1}HNMR (δ, DMSO d_6)

3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.10 (s, 3 H, OCH_3), 6.78 (s, 1H), 7.0 (d, J=8 Hz, H-3',5') and 8.0 (d, J= 8Hz, H-2', 6').

^{13}CNMR (δ, DMSOd_6/$CDCl_3$)

56.3 (q, OCH_3), 59.5 (q, OCH_3), 59.9 (q, OCH_3), 99.0 (d, C-8), 105.8(s, C-10), 115.8 (d, C-3', C-5'), 119.9 (s, C-1') 130.1 (d, C-2', C-6'), 131.7 (s, C-6), 137.5 (s, C-3), 151.8 (s, C-5 or C-9), 151.9 (s, C-9 or C-5), 156.2 (s, C-2), 158.5 (s, C-7), 161.3 (s, C-4'), 178.2 (s>C=O).

Mass (m/e) 344 (M^+) 329, 301, 287, 181, 153, 121.

ACETYLATION OF COMPOUND 'A'

The compound '**A**' (75 mg) was dissolved in pyridine (1.0 ml) and acetic anhydride (1.0 ml) was added to it. The contents were heated on a boiling water bath for four hours under anhydrous conditions and then poured dropwise into ice cold water (100 ml) with constant stirring. A white precipitate so obtained on usual work up and crystallization with methanol gave a colourless compound (**A-A**), m.p. 172-73°. A T.L.C. examination showed it to be a single component.

^{1}HNMR (δ, $CDCl_3$)

2.30 (s, 3H, $OCOCH_3$), 2.5 (s, 3H, $OCOCH_3$), 3.75 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.9 (s, 3H, OCH_3), 6.8 (s, 1H) 7.2 (d, J=8 Hz, H-3',5') and 8.05 (d, J=8 Hz, H-2', 6').

Mass (m/e) 428 (M^+), 386, 371, 344, 329, 326.

STUDY OF THE ACETONE SOLUBLE FRACTION

The solvent was distilled off and the residue (10 gm) was taken up in water (1 lit.). It was filtered to give water soluble and water insoluble fractions. The water soluble fraction, gave an intense yellow colour on exposure to ammonia vapours and a pink colour with Mg/HCl, indicating it to be a flavonoidal glycoside.

HYDROLYSIS OF WATER SOLUBLE FRACTION

It was hydrolysed with 4% sulphuric acid v/v first by heating on a boiling water bath for two hours and then by refluxing on a wire gauze for another two hours. The hydrolysed product was

allowed to cool to room temperature and filtered. A dark black precipitate (4.0 gm) was obtained, which was washed with a large volume of water till free from acid. On exhaustive extraction with solvent ether and recovery of the solvent, it gave the crude aglycone (1.0 gm).

PURIFICATION OF THE CRUDE AGLYCONE

The above crude aglycone (1.0 gm) was subjected to column chromatography on silica gel and the column was eluted with petroleum ether (500 ml), benzene (500 ml), benzene: ethyl acetate mixtures (3:1 & 1:1, 500 ml each) and finally with pure ethyl acetate (500 ml). Benzene: ethyl acetate (3:1) eluate (500 ml) was evaporated to dryness to give a solid residue (500 mg) which was examined by T.L.C. in toluene: ethyl formate: formic acid (5:4:1) solvent system under U.V. light. It showed two brown spots (Rf. 0.61 and 0.63).

SEPARATION OF COMPOUND 'B' AND 'C' BY PREPARATIVE T.L.C.

This mixture was separated by preparative T.L.C. using toluene: ethyl formate: formic acid (5:4:1) as solvent system. About 150 silica gel G. plates (size 20 cm x 20 cm) were run. The two bands (upper and lower Rf. 0.63 & 0.61) were carefully separated from the plates, and eluted with methanol. The solvent was evaporated off to dryness and the two fractions so obtained were crystallised from methanol to give the compound **'B'** from upper band (m.p. 305-306°, 40 mg) and compound **'C'** from lower band (m.p. 313-14°, 15 mg).

COMPOUND B, m.p. 305-306°

It gave a pink colour with Mg/HCl, an intense yellow colour on exposure to ammonia vapours and green colour with alcoholic $FeCl_3$ solution which indicated its flavonoidal nature. It was identified as isorhamnetin.

SPECTRAL DATA

U.V. (MeOH)

λ_{max}^{MeOH} 255.2, 267.38(inf), 326.75(inf), 371.2 nm.

λ_{max}^{NaOAc} 274.5, 320, 393 nm.

$\lambda_{max}^{NaOAc+Boric\ acid}$ 254.4, 270(inf), 326 (inf), 371.2 nm.

$\lambda_{max}^{AlCl_3}$ 264, 304(inf), 361(inf), 429 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 262,302(inf), 359, 428 nm.

Mass (m/e) 316 (M^+), 288, 273.

ACETYLATION OF COMPOUND 'B'

The compound 'B' (30 mg) was dissolved in pyridine (1 ml) and acetic anhydride (0.5 ml) was added to it. The contents were left overnight at room temperature and then poured into ice cold water with constant stirring. A dull white precipitate separated out, which was filtered, washed with water to remove acetic acid and pyridine and dried. On crystallisation from methanol, it gave colourless needles (**B-A**) (18 mg), m.p. 202-203°.

SPECTRAL DATA

^{1}HNMR (δ, **$CDCl_3$**),

2.3-2.5(bs, 12H, 4x$OCOCH_3$), 3.9 (s, 3H, OCH_3), 6.96 (d, J=2 Hz, H-6), 7.4 (m, 2H, H-8, H-5'), 8.0 (m, 2H, H-2', H-6').

COMPOUND 'C' m.p. 313-14°

It gave a pink colour with Mg/HCl, an intense yellow colour on exposure to ammonia vapours and a green colour with alcoholic $FeCl_3$ solution. By running its T.L.C. alongside with an authentic sample of quercetin, it was found to have the same Rf values. However, due to paucity

of the material its acetyl derivative could not be prepared.

SPECTRAL DATA

U.V. (Methanol)

λ_{max}^{MeOH} 256, 301 (inf), 370.2 nm.

λ_{max}^{NaOAc} 272.5, 327(inf), 390 nm.

$\lambda_{max}^{NaOAc+H_3BO_3}$ 261, 303(inf), 388 nm.

$\lambda_{max}^{AlCl_3}$ 270,302(inf), 333,456.2 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 265,301(inf), 359, 426 nm.

Mass (m/e) 302 (M^+), 274, 152,137.

STUDY OF METHANOL SOLUBLE FRACTION

Recovery of solvent left a dark brown solid (38 gm) which was taken up in water and filtered. The filtrate gave a pink colour with Mg/HCl and an intense yellow colour on exposure to ammonia vapours indicating the presence of glycosidic flavonoids. In addition to these tests, this fraction on shaking with a drop of sodium bicarbonate solution formed an honey comb like frothing which persisted for more than 30 minutes suggesting the presence of saponins.

HYDROLYSIS OF THE FILTRATE

The filtrate was hydrolysed by 4% v/v H_2SO_4 by heating first on a boiling water bath for 2 hours and then completing the hydrolysis by refluxing for another one hour. The aglycone in the

form of black precipitate (6.0 gms) was obtained, which was filtered, washed with water several times till free from acid and dried. In order to fractionate, it was exhaustively extracted successively with chloroform and solvent ether.

STUDY OF THE CHLOROFORM EXTRACT OF THE AGLYCONE

Recovery of the solvent left a semi solid brown residue. A T.L.C. examination in the solvent system (T:E:F; 5:4:1) showed it to be a mixture of flavonoids and triterpenes. All attempts to purify this fraction through crystallisation with methanol did not succeed and hence it was converted into its acetyl derivative.

ACETYLATION

The semi solid brown residue (500 mg) was dissolved in pyridine (2.0 ml) and acetic anhydride (1.0 ml) was added to it. The contents were heated on a boiling water bath for four hours. The reaction mixture was cooled and poured dropwise into ice cold water (300 ml). A brown precipitate separated out which was filtered washed with water and dried. On T.L.C. examination in (T:E:F; 5:4:1) and developing the spots by spraying with 4%, H_2SO_4 and heating the plates at 105° for 30 minutes it was found to be a mixture of three components.

FRACTIONAL CRYSTALLISATION OF THE ACETYL DERIVATIVE AND ISOLATION OF COMPOUND 'D'

The crude acetate was crystallised with methanol containing a few drops of chloroform to give a colourless compound 'D' (100 mg, m.p. 202-3°). It did not show any depression in m.p. on mixed melting with the acetate of compound 'B' m.m.p. (202°), and had the same Rf value on T.L.C. examination (T:E:F; 5:4:1). It was identified as isorhamnetin acetate.

SPECTRAL DATA

^{1}HNMR (δ, **$CDCl_3$**)

2.3-2.5 (bs, 12H, 4x $OCOCH_3$), 3.9 (s, 3H, OCH_3), 6.95 (d, J=2Hz, H-6), 7.4 (m,2H, H-8, H-5'), 8.0 (m, 2H, H-2', H-6').

DEACETYLATION OF THE ABOVE ACETYL DERIVATIVE

The above acetate (50 mg) was refluxed with methanolic hydrochloric acid (25 ml, 4%) on a water bath for four hours. Condenser was removed and half of the solvent was evaporated off. The rest of the reaction mixture was diluted with water. A light brown precipitate was obtained, which was extracted with solvent ether in a separating funnel twice. The two ethereal extracts were combined together and shaken with water to remove acid. The ethereal layer was then dried over sodium sulphate and filtered. The ether was distilled off from the filtrate. A residue in small quantity was obtained which was crystallised with methanol to give a yellow compound, (10 mg, m.p. 302°). It gave a pink colour with Mg/HCl and an intense yellow colour on exposure to ammonia vapours and a green colour with alcoholic $FeCl_3$ solution. By running its T.L.C. alongwith an authentic sample of isorhamnetin, it was found to be identical and did not show any depression in m.p. on mixed melting with isorhamnetin (m.m.p. 302°).

SPECTRAL DATA

U.V. (Methanol)

λ_{max}^{MeOH} 255.7, 267(inf), 326(inf), 371 nm.

λ_{max}^{NaOAc} 274.8, 320, 393 nm.

$\lambda_{max}^{NaOAc+H_3BO_3}$ 255.9, 270(inf), 326(inf), 370 nm.

$\lambda_{max}^{AlCl_3}$ 264, 304 (inf), 361(inf), 429 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 262, 302 (inf), 359, 428 nm.

ISOLATION OF COMPOUND 'E'

The mother liquor after filtering off isorhamnetin acetate (compound **D**) was heavily charcoaled in order to remove flavonoidal constituents and concentrated to a small volume. On leaving at room temperature for 24 hours, it gave a colourless compound (m.p. 283-85°, 75 mg) which gave a violet colour in L.B. test. It was identified as doviscogenin acetate.

STUDY OF THE ETHEREAL EXTRACT OF THE AGLYCONE AND ISOLATION OF COMPOUND 'F'

Recovery of the solvent left a brown solid mass which on repeated crystallisations with methanol gave a yellow crystalline compound, m.p. 304-305° (150 mg). It gave a pink colour with Mg/HCl, an intense yellow colour with ammonia vapours and a green colour with alcoholic $FeCl_3$ solution. By running its T.L.C. alongwith an authentic sample of isorhamnetin, it was found to be identical. By mixed melting with isorhamnetin, it did not show any depression in m.p. (m.m.p. 304-305°).

SPECTRAL DATA

(U.V. Methanol)

λ_{max}^{MeOH} 255.2, 267.3 (inf), 326 (inf), 371.2 nm.

λ_{max}^{NaOAc} 274.5, 320, 393 nm.

$\lambda_{max}^{NaOAc+H_3BO_3}$ 254, 270(inf), 326(inf), 371.2 nm.

$\lambda_{max}^{AlCl_3}$ 264, 304(inf), 361(inf), 429 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 262, 302(inf), 359, 428 nm.

ACETYLATION

The above compound (100 mg, m.p. 304-305°) was dissolved in pyridine (1.5 ml) and acetic anhydride (1.0 ml) was added to it. It was left at room temperature for 24 hours and then poured dropwise into ice cold water. A precipitate so obtained on usual work up and crystallisation with methanol containing a few drops of chloroform gave colourless crystals m.p. 202-03°. On TLC examination alongwith an authentic sample of isorhamnetin acetate, it was found to be identical and on mixed melting with isorhamnetin acetate, it did not show any depression in m.p. (m.m.p. 200-201°).

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CHAPTER - VI

DISCUSSION

STUDY OF THE MALE FLOWERS OF *LUFFA CYLINDRICA* (LINN) M.J.ROEM. SYN.,*LUFFA AEGYPTICA* MILL EX. HOOK F. (N.O. CUCURBITACEAE)

VERNACULAR NAMES

Local Name-Torai, **English**-sponge gourd, vegetable gourd; **Sans**-Rajakoshataki, dirgha, patolika; **Hindi**-Ghiya Tori; **Beng**-Dhundal; **Mar.**-Ghosali; **Guj**-Turia; **Tel.**-Guthibira; **Tam.**-Mozhuku pirkankai; **Kan.**-Tuppahirchai; **Mal.**-Kattu-peechal, **Arabic**-Luff, Luffa; **Brazil**-Bucha dos paulistas; **Chinese**-Szu Kua.

DISTRIBUTION

Sponge gourd is said to be indigenous to India. It is cultivated throughout the greater part of India as well as in Africa and America. It is found wild in North West India,Bihar, Bengal, Sikkim and Assam and also in Madras. Both edible and bitter forms are known.[1]

BOTANICAL DESCRIPTION

It is a large climber with palmately 5-7 angled or lobed leaves: petiole without glands at the apex, plants monoecious (rarely dioecious); male flowers with 5 stamens in 10 to 20 flowers racemes usually crowded near the top of raceme. Female flowers solitary usually from the same axil as the males. Fruit smooth, cylindrical or somewhat trigonous blunt at the end usually 20-50 cm. long. Seeds are black or grey 10x6 mm,much compressed, narrowly winged, smooth or slightly tuberculate.[1-2]

LITERATURE SURVEY

A clear liquid, extracted in Japan from the stem of the plant by making incisions is said to be useful in respiratory complaints. Its leaves are used for the treatment of chronic bronchitis[3]. The tender fruit is considered diuretic and lactagogue. Ripe fruits are used in China after burning and pulverizing as carminative and anthelmintic. The juice of the fruit is purgative. The fruits are mostly used as diuretic and applied to tumours as poultice. The fruit is reported to be oleagenous, laxative,

removes "Vata" and biliousness, and is said to be useful in leprosy (Ayurveda), syphilis, fever, spleen disease, piles, haematuria, and bronchitis.[1,4,5]

The ethanolic extract of the seeds showed fungitoxic activity[6] and their aqueous extract revealed anticancerous activity in transplanted tumours[7]. Mature seeds are bittter, emetic and cathartic. The seed oil may be used as a substitute for olive oil and is said to be useful in skin disease. The bitter seed cake may be used as a manure.[1,4,5] The juice of seedlings showed antifungal activity against *Cephalosporium sacchari* and *Fusarium nivate.*

The saponin-I(**XIX**) isolated from aerial parts of *L. cylindrica* is a health food additive which is effective in controlling obesity and side effects of steroids[8]. A saponin lucyoside from *L. cylindrica* can be used as an antitussive.[9]

Young fruit is edible and used as a vegetable, the dry mature fruit which has interwoven network of fibres is used as skin brush for cleaning purposes.[1]

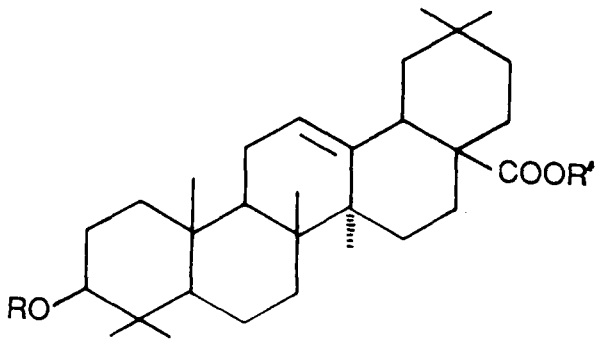
Several investigations carried out in the past on different parts of this plant revealed that all parts are saponin bearing. It is interesting to note that oleanolic acid as sapogenin is a common constituent of all the saponins present.

The composition of the air dried powder of seeds has been reported to have moisture 5.2%, ash 3.9%, fat 38.9% and protein 23.6%. The characteristics of the fat are: n^{30} 1.4793, acid value-1.1, Iodine value-104.3, saponification value 186.6 and the composition is : stearic acid-14.4%, palmitic acid-22.6%, oleic acid-8.4%, linoleic acid-54.5%. The amino acids identified in the protein fractions of seeds are alanine, arginine, aspartic acid, glutamic acid, histidine, lucine, lysine, phenylalanine, proline, serine, tryptophan, tyrosine and valine. The carbohydrate fraction of the seeds contained stachyrose, raffinose and sucrose.[10] Grindley [11] in the analysis of the oil gave the unsaponifiable matter as 1.3% and Pishawikhar and Shah[12] reported the presence of an unidentified product m.p.144.8° in the corresponding fraction.

The structure of the bitter principle in the seeds does not seem to have been fully characterised [13,14]. Rangaswami and Sambamurty[15] reported that a product with the molecular formula $C_{15}H_{24}O_4$, m.p. 182-184°$[\alpha]_D$=40.3°, acetate m.p. 129°, benzoate m.p.130° is responsible for the bitter taste, Fayez and Osman [16] reported that the seeds yielded 39% of an oil and gave

an unsaponifiable fraction 1.4% which was found to contain a considerable amount of an oily product identified as squalene. The presence of such a high squalene content is significant in view of its proven utility as an effective lactogenic agent. A crystalline sterol m.p. 174-5° $[\alpha]_D$=11° was also isolated from the unsaponifiable fraction and was identified as α-spinasterol.

The defatted meal of *L. cylindrica* has been examined by Barua and Bose [17] who isolated a crystalline saponin giving upon acid hydrolysis oleanolic acid and a neutral genin $C_{30}H_{48}O_3$ m.p. 176-177°. The filtrate of the saponin hydrolysate was examined by unidimensional descending paper chromatography. The sugars were identified by comparision as galactose, arabinose, xylose and rhamnose.[18] Later on Varshney and Khan[19,20] reported the presence of a saponin from *L. aegyptica* seeds (black variety) yielding oleanolic acid, gypsogenin and an unidentified neutral genin, the acetate of which had m.p. 262-264.° In 1977 varshney and Beg [21] proposed a tentative structure of saponin as Aegyptinin A and Aegyptinin B. Aegyptinin A on hydrolysis yielded besides oleanolic acid as the genin, glucuronic acid, glucose, arabinose, xylose and rhamnose in a molar ratio of 1:1:2:3:3. Aegyptinin B yielded glucuronic acid, galactose, glucose, arabinose, xylose and rhamnose in molar 3:2:2:1:1 and oleanolic acid as the genin. Attachment of the sugar residue at C-17(COOH) and C-3(OH) of the genin is indicated. Varshney *etal* [22] further elaborated on the structure of aegyptinin A and B. Aegyptinin A(**I**) was established as (I-R-glucuronic acid, glucose, arabinose, xylose, rhamnose, R-arabinose, xylose, rhamnose,RR'-glucuronic acid, glucose, arabinose, xylose, rhamnose(1:1:2:3:3) and Aegyptinin B(**II**) as II-R-glucuronic acid, galactose, glucose, arabinose, xylose, rhamnose(3:1:1:1:1), on the basis of enzymatic and partial alkaline hydrolysis and methylation studies.



Aegyptinin A

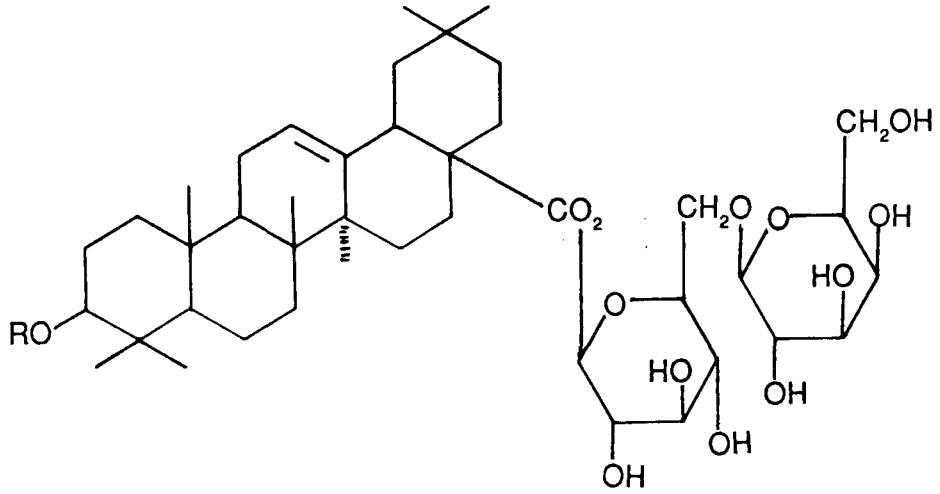
(I)

R = glucuronic Acid, glucose, arabinose xylose, rhamnose.

R'= arabinose, xylose, rhamnose

and

RR' = glucuronic acid, glucose arabinose, xylose, rhamnose(1:1:2:3:3)



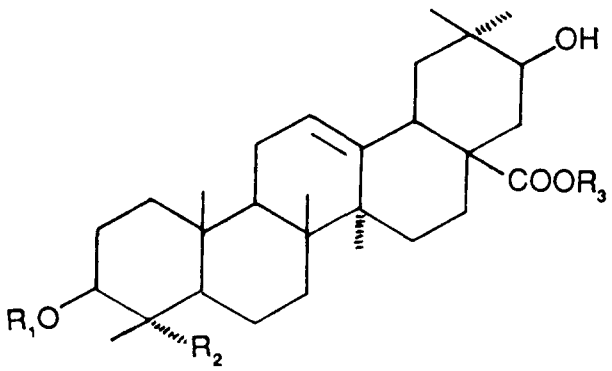
R = glucuronic acid, galactose, glucose, arabinose, xylose rhamnose(3:1:1:1:1).

Aegyptinin B(II)

Miss Ganju & Miss. B. Puri[23] in 1959 reported that the fruits of *Luffa cylindrica* did not show the presence of bio-flavonoids.

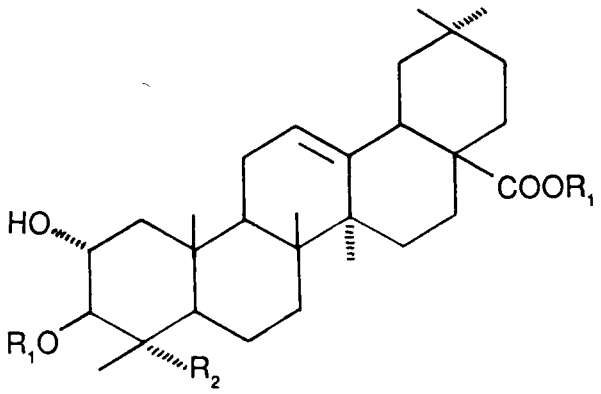
The chemical examination of its leave was first undertaken by a Chinese scientist[3] in 1980, who reported presence of oleanolic acid, palmitic acid, oleanolic acid-3 glucosyl-28-diglucoside in the leaves.

A detailed study on the saponin constituents of the herb was carried out by Japanese workers. Takemato *etal* [24] in 1984 isolated several new saponins named as lucyoside-A(**III**),-B(**IV**),-C(**V**),-D(**VI**),-E(**VII**),-F(**VIII**),-G(**IX**) AND-H(**X**) besides known gensenosides-Re(**XI**) and Rg(**XII**) which were assigned structures on the basis of mass and NMR data.



(**III**) R_1R_3 = glc.,R_2= CH_2OH

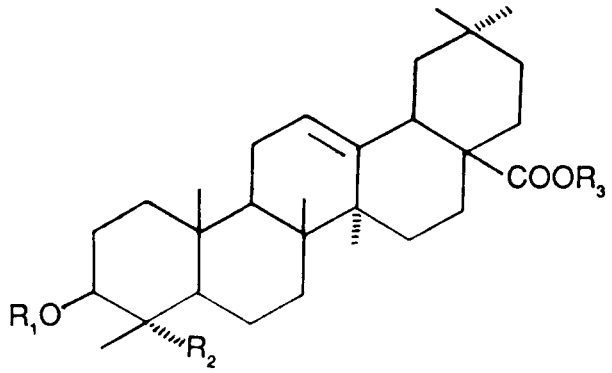
(**V**) R_1R_3 = glc.,R_2= CH_3



(IV) R_1 = glc.,R_2 = CH_2OH

(VI) R_1 = glc.,R_2 =CHO

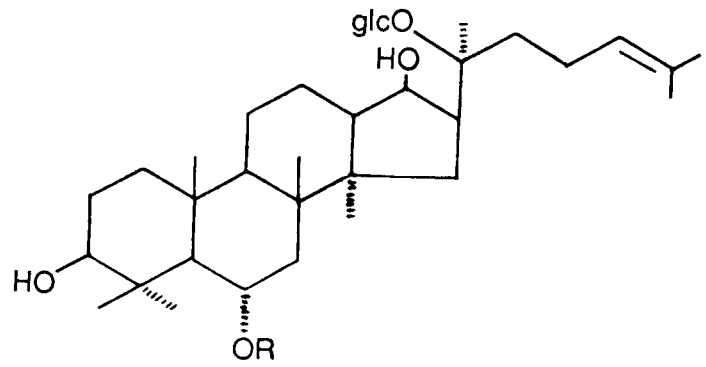
(IX) R_1 = glc.,R_2 = CH_3



(VII) R_1R_3 = glc.,R_2= CH_2OH

(VIII) R_1R_3 = glc.,R_2= CHO

(X) R_1R_3 = glc.,R_2= CH_3

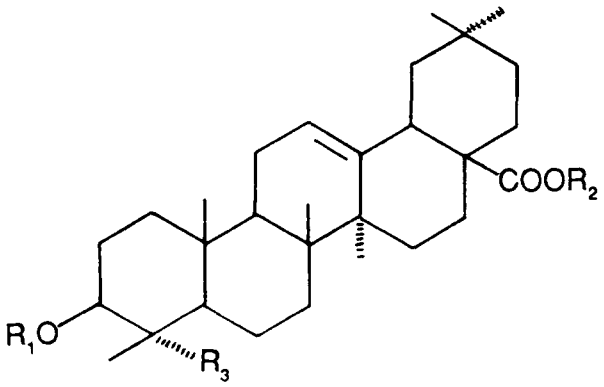


(XI) R = glc. 2 rham.

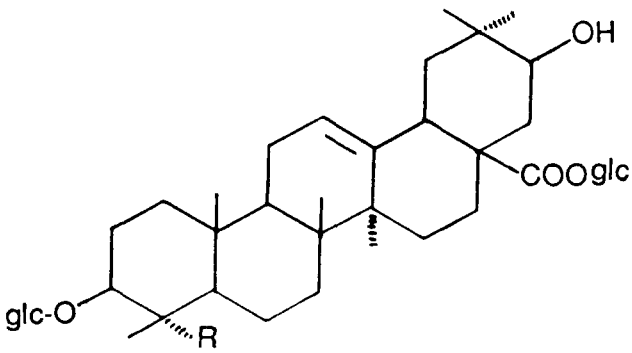
(XII) R = glc.

Takemato[25] carried out their studies further on its fruits and reported isolation and characterisation of saponins named as Lucyoside-A(**III**),-E(**VII**),-F(**VIII**), 3-O-β-D-glucopyranosyl hederagenin (**XIII**), β-O-β-D-glucopyranosyl oleanolic acid (**XIV**), Lucyoside-J(**XV**),-K(**XVI**)-L(**XVII**) and-M(**XVIII**).

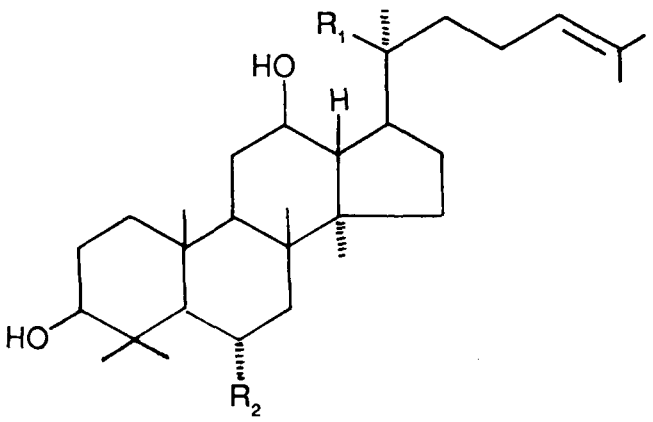
Osaka and Kenkyushkok [8] in 1985 isolated a new saponin named as saponin-I (**XIX**), its structure was established, on the basis of spectral data.



	R_1	R_2	R_3
(XVI)	glc	H	CHO
(XVII)	glc^{2}glc	-glc	CHO
(XVIII)	glc^6 Ac	-glc	CHO
(XIII)	glc	H	CH_2OH
(XIV)	glc	H	CH_3



(XV) R = CHO



R_1 = O- glucose

R_2 = O-glucose or glucose rhamnose.

(XIX)

As no work seems to have been done on the flavonoidal constituents of this plant hence investigations were undertaken.

PRESENT WORK

Fresh male flowers were collected from town, Bilari, Distt. Moradabad(U.P) during the months of June & July and dried under shade. The dried flowers were exhaustively extracted with ethanol. The ethanolic concentrate was successively extracted with petroleum ether and acetone. The acetone fraction after evaporation to a small volume was diluted with ether which gave a brown precipitate. The brown precipitate was filtered off. The filtrate was transferred into separating funnel and shaken with water. The ethereal layer was removed and dried over anhydrous sodium sulphate. After removing inorganic salts, the solvent was evaporated off and the residue exhaustively extracted with benzene. The benzene insoluble residue on repeated crystallisation from methanol gave a T.L.C. pure yellow crystalline compound (m.p > 330°) which gave positive tests for flavonoids.

CHARACTERIZATION OF THE ABOVE COMPOUND

The U.V. spectrum of the compound showed two bands at 268.8 nm (Band II) and 337.6 nm (Band I) with an inflexion around 297 nm. The shifts in the Band I on addition of $AlCl_3$ and Band

II on addition of NaOAc indicated hydroxyls at C-5 and C-7. Absence of shift with H_3BO_3+NaOAc ruled out any catecholic unit. This behaviour was similar to that noted in apigenin (page 31).

In the mass spectrum the molecular ion peak located at m/e 270 as expected for apigenin. Loss of CO led to a peak at m/e 242. RDA cleavage gave the A-ring fragment at m/e 152 (and 153) and the B-ring fragment at m/e 118. The acylium ion fragment originating from B-ring was located at m/e 121. These are also similar to that observed in apigenin (page 31).

These findings are consistent with the formulation of the compound as **apigenin**. The mass spectral fragmentation pattern of the compound is shown in the Chart (page 32).

EXPERIMENTAL

STUDY OF THE MALE FLOWERS OF *LUFFA CYLINDRICA* (LINN)M.J. ROEM SYN.,*LUFFA AEGYPTICA* MILL EX. HOOK F. (N.O. CUCURBITACEAE)

Fresh male flowers were collected from the vegetable fields in town Bilari(Moradabad) U.P. India. During the months of June and July and dried under shade.

EXTRACTION

The dry flowers (600 gm) were exhaustively extracted with hot ethanol three times (3x4 lit). All the ethanolic extracts were combined together. Recovery of the solvent under reduced pressure left a dark brown residue (40 gm). The alcoholic concentrate was extracted successively three times with petroleum ether (60-80°) and three times with acetone.

ISOLATION OF THE COMPOUND APIGENIN

The acetone extract was concentrated to a small volume and diluted with 500 ml of ether. A dark brown precipitate so obtained was filtered off. The filtrate was transferred into separating funnel and shaken with water. The ethereal layer was removed and dried over anhydrous sodium sulphate. The inorganic salt was filtered off and the solvent evaporated to dryness. The residue was exhaustively extracted with benzene. The benzene insoluble residue on crystallization from methanol gave yellow compound m.p. >330° (10 mg). On T.L.C examination in various solvent systems (T:E:F; 5:4:1), ($CHCl_3$; MeOH; 95:5) and (Chloroform: toluene: acetone; 8:5:7), it was found to be a single entity. It gave a pink colour with Zn- HCl, a reddish brown colour with alcoholic $FeCl_3$ solution, an intense yellow colour on exposure to ammonia vapours and a brown flourescence under U.V. light.

Due to paucity of the material its acetyle derivative could not be prepared.

SPECTRAL DATA

U.V. (METHANOL)

λ_{max}^{MeOH} 268.8, 297(inf), 337.6 nm

λ_{max}^{NaOAc} 275.2, 300.8, 385.8 nm.

$\lambda_{max}^{NaOAc + H_3BO_3}$ 268.8, 302(inf), 338.4 nm

$\lambda_{max}^{AlCl_3}$ 276.8, 301.6, 354.6, 382.4 nm

$\lambda_{max}^{AlCl_3 + HCl}$ 277.6, 300.4, 342.4, 376.6 nm

MASS (m/e)

270(M^+), 242, 153, 152, 121, 118.

On the basis of above spectral data, co-T.L.C. examination and m.m.p., it was identified as apigenin.

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CHAPTER - VII

DISCUSSION

STUDY OF THE WHOLE PLANT OF *CORONOPUS DIDYMUS* LINN (N.O CRUCIFERAE)

C. didymus is commonly known as "Jangli Hala" or "Panacholi". It is a winter season weed and is highly variable in size and appearance. It is found in fields and open places on moist sandy soil and is abundantly available in winter. In summer and early rainy season it becomes rare. The plants that survive till early summer become somewhat woody[1]

BOTANICAL DESCRIPTION

It is a prostrate or an ascending, branching leafy, rather hispid herb often forming a rosette. Leaves are pinnatifid or pinnati- partite; lobes spreading. Flowers are pale green, small, sometimes apetalous, diandrous. Pods are 1x2 mm in size; separating into 2; indehiscent, reticulate lobes, seeds about 1x1 mm, brown.[1]

LITERATURE SURVEY

A survey of literature showed that its seeds contain benzyl isothiocyanate, benzyl cyanide [2,3] and fatty acids [4] (Palmitic, oleic, linoeic and linolenic acids). Leaves on preliminary examination have been reported to give positive tests for flavonoids and alkaloids only [5,6]. However, no systematic and detailed work seems to have been done on these constituents and hence the present studies were undertaken.

PRESENT WORK

The plant material was collected from the Campus of Hamdard Nagar, New Delhi and dried under shade. The dried material was exhustively extracted with 95% ethanol. Recovery of solvent left a greenish viscous mass which was treated with petroleum ether (60-80°) and $CHCl_3$ in order to remove petrol and chloroform soluble products. The insoluble residue was extracted five times with solvent ether.All the ethereal extracts were combined together. Recovery of ether left a brown semisolid mass which on crystallisation from methanol gave a T.L.C. pure yellow crystalline compound, m.p. > 330°. It gave positive tests for flavonoid. On acetylation with pyridine and acetic anhydride, it gave a colourless acetyl derivative, m.p. 220-21.°

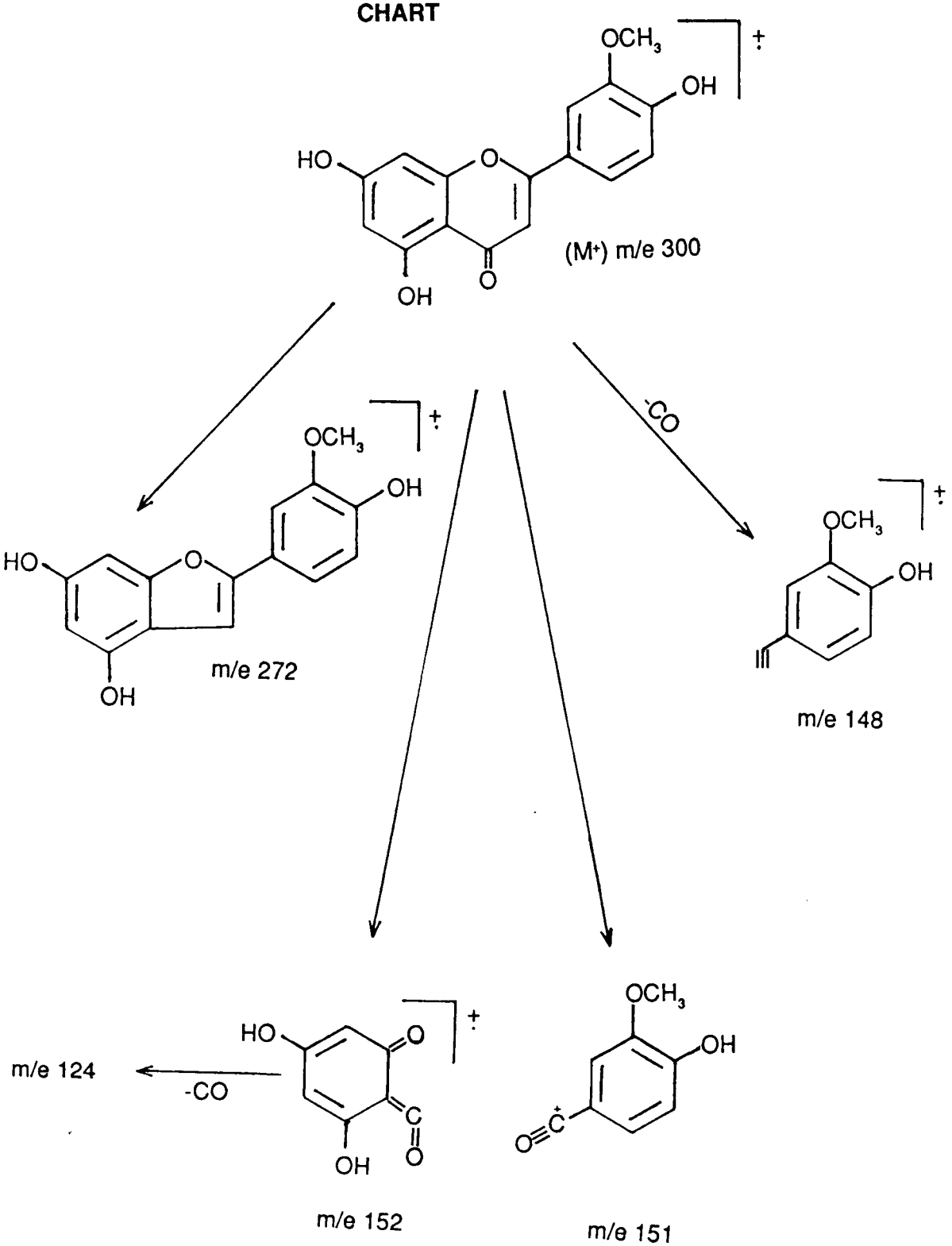
The U.V. spectrum of the parent compound, m.p. >330° showed two maxima at 268 nm (band II) and 344 nm (Band I) with an inflexion around 295 nm. Addition of NaOAc shifted band II to 276 nm, thus suggesting a free 7-OH group. In $NaOAc/H_3BO_3$, the spectrum reverted to the parent compound spectrum in having the maxima at 269 and 347 nm, thus suggesting the absence of any catecholic unit.

Addition of $AlCl_3$ replaced band I with a double maxima at 360 and 388 nm, thus suggesting the presence of a free 5-OH group. Addition of HCl to $AlCl_3$ containing solution did not produce any drastic change which also supported the absence of catecholic system. The above data led to the conclusion that the compound is a 5,7- dihydroxy flavonoid which does not have free catechol unit in the side phenyl. However, the possibility of protected catecholic system was not ruled out.

The N.M.R. spectrum of the above acetate m.p.220-21° showed a singlet for two acetoxyls at δ 2.5 and another singlet for the third acetoxyl at δ 2.6. There was a methoxyl singlet at δ 4.0. In the aromatic region the H-3 appeared as a singlet at δ 6.7 and H-6 appeared as a doublet (J = 2Hz) at δ 6.95. The remaining protons namely H-8, H-2', 6', 5', all appeared as a multiplet between δ 7.3 and δ 7.6. Thus the parent compound could be a monomethyl ether of tetrahydroxy flavonoid. The presence of H-6 appearing as a meta coupled doublet indicated that the compound carries 5,7-dioxygenation. The chemical shifts of the H-6 and H-8 are more closely resembling 5,7-diacetoxy flavonoids rather than 5-acetoxy, 7-methoxy systems. Hence it appears that the methoxyl group is present in the side phenyl.The probable structure could be luteolin 3'-methyl ether (Chrysoeriol) or 4' methyl ether (Diosmetin). A comparison of physical data of chrysoeriol (5,7,4'-trihydroxy 3'-methoxy flavone) with this compound confirmed it to be chrysoeriol.

A further support to the above findings was obtained by the mass spectral studies (**Chart**). In the mass spectrum the molecular ion peak was observed at m/e 300 suggesting the mol. formula $C_{16}H_{12}O_6$. Loss of CO from the molecular ion peak typical of flavonoids was observed in this case leading to a peak at m/e 272. The RDA fragmentation gave two peaks at m/e 152 originating from ring-A and at m/e 148 originating from ring-B. These data suggest that the compound must be carrying two hydroxyls in ring-A and one hydroxyl and one methoxyl in ring-B. Consistent with these inferences the substituted benzoyl ion originating from ring-B was observed at m/e 151. These studies also led to the conclusion that this compound is **chrysoeriol**.

CHART



EXPERIMENTAL

STUDY OF THE WHOLE PLANT OF *CORONOPUS DIDYMUS* LINN (N.O. CRUCIFERAE)

Fresh plant material was collected from Hamdarad Nagar Campus, New Delhi India, during the month of September. It was chopped into small pieces and dried under shade.

EXTRACTION

Shade dried whole plant material (1 kg) was exhaustively extracted with ethanol 95%. Recovery of solvent left a viscous greenish mass (150 gms), which was treated successively with petroleum ether (60-80°) and $CHCl_3$ under boiling conditions in order to remove petrol & $CHCl_3$ soluble products. The residue left after petrol & chloroform extraction was exhaustively extracted with solvent ether five times. All the ethereal extracts were combined together. Recovery of ether left a brown semi solid mass (5.0 gm).

ISOLATION OF PURE COMPOUND

The semi solid mass so obtained was dissolved in methanol & left at room temperature for crystallisation. It gave a yellow crystalline compound which on repeated crystallisation with methanol yielded a microfine crystalline compound (150 mg), m.p. > 330°. On T.L.C.(silica gel) examination in various solvent systems (T:E:F; 5:4:1, $CHCl_3$: MeOH, 95:5, chloroform: toluene: acetone; 5:8:7), it was found to be a single compound. It gave a pink colour with Zn-HCl, a green colour with $FeCl_3$ solution, an intense yellow colour on exposure to ammonia vapours and showed brown flourescence under U.V. light.

SPECTRAL DATA

U.V. (MeOH)

λ_{max}^{MeOH} 268, 295(inf), 344 nm.

λ_{max}^{NaOAc} 276, 295(inf), 394. nm.

λ_{max}^{NaOAc} + Boric acid 269, 295(inf), 347 nm.

$\lambda_{max}^{AlCl_3}$ 273, 295(inf), 360, 388 nm.

$\lambda_{max}^{AlCl_3+HCl}$ 274, 295(inf), 360, 388 nm.

MASS (m/e)

300 (M^+), 272, 152, 151, 148, 124.

ACETYLATION

The above compound (100 mg) was dissolved in pyridine (2 ml) and acetic anhydride (1.5 ml) was added to it. The contents were left overnight at room temperature and then poured into ice cold water with constant stirring. A precipitate separated out, which was filtered, washed with water in order to remove acetic acid and pyridine and dried. On crystallisation from methanol it gave a T.L.C. pure colourless compound (75 mg), m.p. 220-21°.

SPECTRAL DATA

^{1}HNMR (δ, $CDCl_3$)

2.5(s,6H, 2x$OCOCH_3$), 2.6(s, 3H, $OCOCH_3$), 4.0(s, 3H, OCH_3), 6.7(s, 1H, H-3), 6.95(d, J=2 Hz, H-6), 7.3 -7.6(m, 4H, H-2', 5', 6', H-8).

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SUMMARY

SUMMARY

Plants have always been a common source of medicaments either in the form of traditional preparations or as pure active principles. It is mainly during the last 100 years that some of the active ingredients present in herbal prescriptions have been isolated and introduced into "**MODERN MEDICINE**". There are atleast **119 distinct chemical substances** derived from plants that can be considered as important drugs currently in use. A few of the drugs are simple synthetic modifications of naturally occurring substances. In some instances, the natural products have recently been replaced by commercially available synthetic products. Thus drugs derived from plants still occupy an important position.

In the present studies we have carried out systematic chemical investigation of seven important indigenous medicinal plants and isolated and elucidated the structures of a number of compounds. These products may be helpful to other researchers who are mainly concerned with the biological and clinical aspects of the herbal drugs.

The present thesis is based on **seven chapters**. *Each chapter is devoted to one particular medicinal plant on which the present detailed chemical investigations have been carried out. In each chapter, area of distribution of the plant alongwith its different vernacular names, therapeutic uses of the plant in different traditional systems of medicine and an exhaustive review of the scientific work reported in literature have been given.*

In the beginning of the thesis in the part of "**Introduction**", *the importance of medicinal plants in general has been highlighted and the biological importance of the two groups of compounds, namely triterpenoids and flavonoids has been reviewed, the reason being that the compounds which we isolated and characterised are mostly flavonoidal and triterpenic in character.*

CHAPTER-I

STUDY OF THE WHOLE PLANT OF *CORCHORUS DEPRESSUS* (LINN) SYN., *C. ANTICHORUS* (RAEUSCH) OR *ANTICHORUS DEPRESSUS* (LINN) (N.O. TILIACEAE)

The dried plant material was chopped into small pieces and exhaustively extracted with boiling ethanol. The ethanolic concentrate on leaving in a refrigerator deposited a solid which was filtered and coded as "A". The filtrate was evaporated to dryness and the residue extracted

successively with(i) petroleum ether(60-80°) to give E-1; with (ii)chloroform to give E-2 and finally with (iii) acetone to give E-3. E-2 was further resolved into neutral and acidic fractions (F-1) and (F-2) respectively. E-3 was dissolved in water and filtered to give the water soluble(W-S) and water insoluble (W-INS)fractions. The water insoluble fraction (W-INS) was dissolved in boiling ethyl acetate. After concentrating the solution to a small volume and leaving in the fridge, a solid "B" separated out which was filtred. The filtrate was evaporated to dryness and coded as "BM". Usual work up and purification of all the above extracts/fractions gave different compounds which are listed in the following Table-I

TABLE-I

S.No.	Fraction/Extract	Compound
1	"A"	2α, 3ß, 20ß-trihydroxy-urs-Δ^{12}-ene,24, 28 dioic acid.
2.	E-1	ß-sitosterol
3.	F-1	ß-sitosterol-ßD-glucoside
4.	F-2	2α,3ß,20β-trihydroxy-urs-Δ^{12}-ene 24, 28-dioic acid.
5.	W-S	Corchorenic acid (2α,3ß-dihydroxy-urs-$\Delta^{12,20}$-diene 24,28 dioic acid
6.	B	Depressin (2α,3ß,20ß-trihydroxy-urs-Δ^{12}-ene-24,28 dioic acid 24-ßD-galactoside
7.	BM	(i) Apigenin (ii) Luteolin

Chorchorenic acid and **Depressin** are **new compounds** and are being reported for the **first time.**

CHAPTER-II

STUDY OF THE LEAVES OF *ICHNOCARPUS FRUTESCENS* (R.Br) SYN., *APOCYNUM FRUTESCENS* (LINN) (N.O. APOCYNACEAE)

The dried leaves were exhaustively extracted with ethyl alcohol. The ethanolic concentrate was extracted with petroleum ether(60-80°) to give fraction (F-1). The petrol insoluble part was taken up in water and separated into water insoluble fraction (F-2) and water soluble fraction (F-3). The fraction (F-2) was extracted successively with chloroform and solvent ether. The water

soluble fraction (F-3) was subjected to liquid-liquid extraction with petrol, solvent ether, ethyl acetate and butanol successively. The petrol and ether extracts (liquid-liquid extraction) did not give any appreciable quantities of the products and were not studied further. Other fractions on purification gave different compounds which are listed in the following Table-II.

TABLE-II

S.No..	Fraction	Compound
1.	F-1	Ursolic acid
2.	F-2(Chloroform)	Ursolic acid
3.	F-2(Ether)	Kaempferol
4.	F-3(Ethyl acetate)	Kaempferol 3-galactoside
5.	F-3(Butanol)	Mannitol

CHAPTER-III

STUDY OF THE AERIAL PARTS OF *SISYMBRIUM IRIO* (LINN) (N.O.CRUCIFERAE)

The dried plant material (aerial parts) was exhaustively extracted with ethanol and the ethanolic concentrate was extracted with petroleum ether(60-80°) to isolate the petrol soluble products. The petrol insoluble residue was taken up in water and filtered to separate into water soluble and water insoluble fractions. The quantity of water insoluble fraction was too less to be studied further. The water soluble fraction was hydrolysed by dilute acid to give an aglycone. All these fractions were purified to give pure products which are listed in the following Table-III.

TABLE-III

S.No.	Fraction	Compound
1.	Petrol soluble fraction	(i) ß-sitosterol (ii) ß-sitosterol glucoside
2.	Aglycone fraction	(i) Isorhamnetin (ii) Quercetin

CHAPTER-IV

STUDY OF THE FLOWERS OF *ACACIA LEUCOPHLOEA* (ROXB) WILLD SYN., *MIMOSA LEUCOPHLOEA* (ROXB) (N.O. MIMOSACEAE)

Air dried flowers were exhaustively extracted with ethanol and the ethanolic concentrate on extraction with petroleum ether(60-80°) gave a petrol soluble fraction PET-S. The residue left after petrol extraction was dissolved in water and filtered to give water soluble(W-S) and water insoluble (W-INS) fractions. The water insoluble fraction (W-INS) could not be purified inspite of repeated attempts and hence further studies were abandoned. The water soluble fraction(W-S) was subjected to liquid-liquid extraction with petrol(60-80°), solvent ether, ethyl acetate and butanol. The petrol(60-80°), and ether extracts (liquid- liquid extraction) did not yield any appreciable quantity of products to study further. Purification of petrol soluble fraction and products obtained by liquid- liquid extraction gave different compounds which are given below in Table-IV.

TABLE-IV

S.No.	Fraction	Compound
1.	PET-S	(i) Behenic ester (ii) ß-sitosterol
2.	W-S(Ethyl acetate)	Quercetin 3-glucoside
3.	W-S(Butanol)	Mannitol

CHAPTER-V

STUDY OF THE FLOWERS OF *DODONAEA VISCOSA* (LINN)(N.O. SAPINDACEAE)

Fresh flowers were exhaustively extracted with ethyl alcohol. The ethanolic concentrate was successively extracted with petroleum ether (60-80°), chloroform, acetone and methanol. The acetone fraction was dissolved in water and filtered. The filtrate on acid hydrolysis gave an aglycone. Similarly methanol extract was dissolved in water and filtered. The aqueous solution on acid hydrolysis gave an aglycone which was successively extracted with chloroform and solvent ether. Some of these fractions on purification gave different pure compounds which were characterised and are given in the following Table-V.

TABLE-V

S.No.	Fraction	Compound
1.	Chloroform extract of the ethanolic concentrate	Penduletin
2.	Acetone-extract (Hydrolysis product)	i) Isorhamnetin ii) Quercetin
3.	Methanol extract (Hydrolysis product)	
	(a) Chloroform soluble part	i) Isorhamnetin ii) Doviscogenin(3ß, 15α,21ß,22α 28-pentahydroxy-16α *angeloyloxy*-Δ^{12}-*oleanene*
	(b) Ether soluble part	(i) Isorhamnetin

CHAPTER-VI

STUDY OF THE MALE FLOWERS OF *LUFFA CYLINDRICA* (LINN) M.J. ROEM SYN., *LUFFA AEGYPTICA* MILL. EX.HOOK F.(N.O. CUCURBITACEAE)

The dried flowers were exhaustively extracted with ethyl alcohol. The ethanolic concentrate was successively extracted with petrol (60-80°) and acetone. The acetone extract on purification gave apigenin.

CHAPTER-VII

STUDY OF THE WHOLE PLANT OF *CORONOPUS DIDYMUS* (LINN) (N.O. CRUCIFERAE)

Shade dried plant material was exhaustively extracted with ethanol. The ethanolic concentrate was successively extracted with petroleum ether(60-80°), chloroform and solvent ether. The solvent ether extract on purification gave a crystalline compound which was identified as chrysoeriol.